THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Joe Mymryk
DEPARTMENT	Oncology, Cancer Research Laboratories, LRCP
ADDRESS	790 Commissioners Rd. E., Room A4-833
PHONE NUMBER	519-685-8600 x53012
EMERGENCY PHONE NUMBER(S)	519-690-0298
EMAIL	jmymryk@uwo.ca

Location of experimental work to be carried out: Building(s): LRCP Room(s) A4-833, A4-824, A4-826, A4-910

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: Canadian Institute of Cancer Research (CIHR); NSERC
GRANT TITLE(S): Molecular Genetic Analysis of E1A Function, New targets of the human papillomavirus E7 oncoprotein, Identification and analysis of non-conventional nuclear import signals, Human cytomegalovirus tropism for dendritic cells and fibroblasts.

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Jailal Ablack	jnablack@uwo.ca	May 2002
Greg Fonseca	gfonseca@uwo.ca	Sept. 2007
Biljana Todorovic	btodorov@uwo.ca	Oct. 2008
Matt Miller	mmille24@uwo.ca	Sept. 2006
Mike Cohen	mcohen8@uwo.ca	Sept. 2008
Nicole Bindseil	nbindsei@uwo.ca	March 2008

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

Our laboratory is certified as Level 2 lab under Biosafety Approval Number BIO-LRCC-0012.

Adenoviruses are used in the lab and most were obtained from Dr. Stan Bayley, McMaster University upon his retirement 13 years ago. These viruses contain deletion with E1A, E1B or both. All viruses are attenuated as they were constructed in the dl309 background (Bette *et al.* 1995 Virus Res. 39:75-82) and thus contain a deletion within the E3 region such that it does not express the E3 14.7K, 14.5K and 10.4K proteins that block efficient evasion of the host immune response. All new construction involves replacing wild type E1A with mutant versions and uses a system described in McGrory et al. 1988 Virology 163:614-617 that is sold commercially by Microbix Biosystems Inc. (Toronto, ON). Viral waste (liquid or solid) is first autoclaved and then disposed of in biohazard waste containers.

The yeast *Saccharomyces cerevisiae* (baker's/brewer's yeast) is used extensively in the laboratory for yeast two-hybrid tests and other genetic interaction studies. Any liquid waste generated by growing yeast cultures is bleached and subsequently disposed of. Solid waste is directly disposed of in biohazard waste containers.

For cloning applications we used the DH5α strain *of E.coli,* bacteria purchased from Invitrogen. Several other strains of *E.coli* are used for expression of recombinant protein (BL21) or generation of recombinant baculovirus (DH10b) Any liquid waste generated by growing bacterial cultures is bleached and subsequently disposed of. Solid waste is directly disposed of in biohazard waste containers.

Plasmids used for cloning have originally been purchased from different suppliers. Modifications have been made to these and at present our laboratory has 3800+ different constructs. The original plasmids are modified by insertion of genes of interest. In our laboratory there are four major projects. Two involve the human adenovirus E1A gene, while the third involves human papillomavirus E7, while the fourth involves the human cytomegalovirus US9, UL19 and UL148 genes (please see research summary). The newly generated plasmids either have different C or N-terminal tags added to cDNA of interest to allow distinction from the native protein for in overexpression studies. Commonly we also make truncations to the cDNA of interest and clone it into different plasmid backbones (application dependant transient/stable transfection, protein expression) so that different functional protein regions can be identified and protein interactions tested. We often resort to mutational studies where binding sites are mutated and downstream changes observed. Any plasmid waste generated is disposed of in biohazardous waste collected by Stericycle, Inc. on daily basis.

Cell lines listed under section 2.3 have all been obtained through a commercial supplier (see attached MSDS). The only modifications made to these cell lines are by transfection of the previously mentioned plasmids so that the cell line would overexpress tagged, mutated or truncated proteins allowing us to study the importance of the proteins under study. Cell line generated liquid waste is bleached and disposed of while solid waste is disposed in biohazard waste containers.

Please include a one page research summary or teaching protocol.

The four projects in the lab:

1) Genetic Analysis of Adenovirus E1A Function

The protein products of the Early region 1A (E1A) gene of human adenovirus 5 are potent regulators of transcription. E1A is also a growth promoting oncogene. E1A makes many independent connections to the cellular protein interaction network, and many of its targets are key regulators of cell growth and gene expression. These include, but are not limited to pRb, UBC9, CBP/p300 and CtBP. Mechanistically, E1A alters or inhibits the normal function of the cellular proteins that it targets and rewires the cellular protein interaction network. The E1A oncoproteins are powerful tools for investigating mechanisms that contribute to oncogenic transformation, and studies of E1A have contributed much information that is directly relevant to

understanding the molecular basis of cancer.
The E1A proteins target multiple cellular regulatory proteins and these interactions form the molecular basis by which E1A alters growth and gene expression in the host cell. We propose to continue our molecular genetic analysis of E1A function, focusing on aspects of E1A that appear highly relevant to the regulation of gene expression and oncogenic transformation. We will continue to characterize several targets of E1A that we have identified, discover new targets of E1A, elucidate the protein binding motifs mediating these interactions and decipher mechanisms by which E1A utilizes these proteins to reprogram biological processes that are relevant not only to infection, but also the cancer phenotype.

2) New targets of the human papillomavirus E7 oncoprotein
Human papillomaviruses (HPVs) induce warts and have been implicated in causing virtually all human cervical cancers. World-wide, cervical cancers are a leading cause of cancer death in younger women. HPV infection has also been implicated in other anogenital cancers and approximately 20% of oral cancers. Continuous expression of the viral E6 and E7 oncoproteins appears necessary for the maintenance of malignant cell growth. The HPV E7 oncoprotein binds the product of the retinoblastoma susceptibility locus (pRb), which functions as a tumor suppressor. However, research has clearly indicated that pRb is not the only target of E7 in the host cell, nor the sole determinant of E7 function. The interactions of the HPV E7 proteins with cellular regulatory proteins, such as pRb, are key events necessary for the HPV life cycle and the induction of carcinomas. A large body of evidence indicates that interactions with additional cellular protein targets via the unique Conserved Region 3 (CR3) encoded zinc fold motif contribute significantly to the global role of E7 in an HPV infection and HPV induced human cancers. The overall objective of our proposed studies is to characterize in detail the interaction of E7 with these cellular targets and to determine the consequences of these interactions. This information is essential for understanding the mechanisms by which HPVs cause human cancers. Given the critical role for E7 in HPV induced cancer, it could be a promising target for inactivation by small molecule compounds. Our proposed structure/function analysis and detailed study of the fundamental mechanisms behind E7 function will also improve our understanding of eukaryotic gene expression and growth processes that are highly relevant to HPV independent human cancers and are quite likely targeted by other viruses infecting humans.

3) Identification and analysis of non-conventional nuclear import signals.
The human adenovirus type 5 (Ad5) early region 1A (E1A) gene encodes two major proteins of 289 and 243 residues, which differ only by the presence of an internal sequence of 46 amino acids in the larger protein. These multifunctional proteins influence a variety of transcriptional and cell cycle events by interacting with a number of cellular regulatory factors in the nucleus. The Ad5 E1A proteins contain a well characterized monopartite NLS (KRPRP) located near the C-terminus. However, this signal is not conserved in the E1A proteins of many other human adenoviruses, which also lack any other canonical NLSs. A second nuclear import signal, which is unique to the Ad5 E1A 289 residue protein, consists of the sequence FV(X)26MXSLXYM(X)4MF (where X is any amino acid). This sequence does not resemble other known NLSs, appears to be regulated developmentally, and moreover, is not conserved in the E1A sequences of other adenovirus serotypes. Additional regions of Ad5 E1A can also mediate nuclear import, suggesting that E1A contains multiple non-canonical NLSs that function via unknown pathways. As such, the E1A proteins provide an excellent model system to identify and characterize novel mechanisms that contribute to nuclear localization.

4) Human cytomegalovirus tropism for dendritic cells and fibroblasts.
The exceptionally broad tissue tropism of human cytomegalovirus (CMV) contributes greatly to the severe multi-organ disease caused by this virus in immunocompromised individuals. The molecular mechanisms mediating viral entry, persistence and modulation of host response in such a variety of different cell types are still ill defined. Our goal is to reveal the identity and mechanism of action of viral factors contributing to susceptibility or resistance of different cell types to CMV infection. Viral proteins resident in the mitochondria will be identified and characterized.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? X YES O NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES X NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E.coli (DH5α, DH10Bac & BL21)	O Yes X No	O Yes X No	O Yes X No	2L	Invitrogen	X 1 O 2 O 2+ O 3
Human Adenovirus type 5 (*dl*309)	O Yes X No	O Yes X No	O Yes X No	0.1L	Dr. Stan Bayley, McMaster University (See MSDS from Public Health Agency of Canada and ATCC for wildtype hAdV5)	O 1 x 2 O 2+ O 3
Saccharomyces cerevisiae	O Yes X No	O Yes X No	O Yes X No	2L	Open Biosystems	X 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? YES X NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture: **N/A**

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes O No		Not applicable

Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

N/A

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	x Yes O No	HeLa, MCF7, 293, 293T, HT1080, A549, U2OS, IMR-90, WI-38, SAOS-2, Caski, HepG2, KB, C33A	ATCC
Rodent	x Yes O No	MEF, 3T3	ATCC
Non-human primate	X Yes O No	CV-1, COS-1, COS-7, Vero	ATCC
Other (Insect cells)	X Yes O No	SF9, SF21	ATCC, Invitrogen

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 X 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES x NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? x YES NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
DH5α	*The lab has about 3800+ different plasmids on hand. Most commonly used backbones are pEGFP, pcDNA3.1, pGEX4T1,*	*Original constructs have been purchased from various companies, including Promega, Invitrogen,*	*Adenovirus E1A and its cellular targets.* *Human papillomavirus E7 or its cellular targets.*	*Most changes done are to be able to generate a tagged protein so that it can be distinguished from the native protein in Tissue culture cells or to generate a tagged*

	pACTII, pFastBac1, etc. Backbone used is highly dependant on the application. Transient transfection, yeast two-hybrid testing, stable cell line generation or protein expression.	*Clontech and GE Healthcare.*	*Human cytomegalovirus US9, UL19 or UL148 and their cellular targets*	*protein in bacteria for subsequent use. Most constructs contain different subregions of the proteins and these are used to look at changes in interaction with other known interacting proteins.*

* Please attach a Material Data Sheet or equivalent if available.
** Please attach a plasmid map.

4.3 Will genetic modification(s) involving viral vectors be made? X YES, complete table below O NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Human adenovirus type 5	*pXC1, JM17*	*Microbix Biosystems Inc. 115 Skyway Ave Toronto, Ontario, Canada M9W 4Z4 1-800-794-6694 1-416-234-1624 Fax: 416-234-1626 www.microbix.com*	*E1A*	*Loss of function, impaired growth*
Baculovirus	*pFastBac1*	*Invitrogen*	*GCN5 (histone acetyltransferase)*	*Expression of protein for biochemical analysis*

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ X NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ X NO
- SV 40 Large T antigen O YES X NO
- E1A oncogene X YES O NO
- Known oncogenes O YES, please specify _____ X NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ XNO

4.5 Will virus be replication defective? O YES X NO

4.6 Will virus be infectious to humans or animals? X YES O NO

4.7 Will this be expected to increase the containment level required? O YES X NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES X NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O YES X NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____

6.5 Will the agent(s) be shed by the animal: O YES X NO, please justify:

_____

_____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES X No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES X NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? O YES X NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____

9.7 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

10.0 Plants

10.1 Do you use plants? O YES X NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree…)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____ X NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

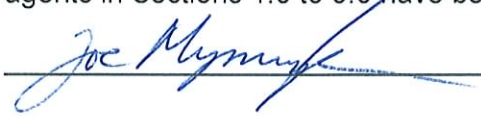
11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE 

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 x 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?
X YES, permit # if on-campus BIO–LRCC- 0012 ✓ Certified Level 2 on July 15, 2009 by Gail Ryder. Gail Ryder
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE Joe Mymryk Date:2010-10-07

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

N/A

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

-Wash the site of injury well under running water
-see Occupational health and safety staffed with Registered Nurses for medical help and to file an incident report
-After hours, go to Emergency

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE:_____
Date:_____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Gail Ryder
Date: October 21, 2010

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

invitrogen™

Material Safety Data Sheet

Revision Date: 20-Sep-2005

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 18258
Product name ME DH5A COMPETENT CELLS

MSDS

Contact manufacturer
INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
dimethylsulfoxide	67-68-5	3-7

3. HAZARDS IDENTIFICATION

Emergency Overview

Irritating to eyes. Irritating to skin. Components of the product may be absorbed into the body through the skin.

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	Irritating to eyes.
Skin	Irritating to skin. Components of the product may be absorbed into the body through the skin.
Inhalation	May cause irritation of respiratory tract.
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — Eyes. Skin.

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material

7. HANDLING AND STORAGE

Handling	Avoid contact with skin and eyes.
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
dimethylsulfoxide	-	-	-	-

Engineering measures — Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form — Liquid

Important Health Safety and Environmental Information

Boiling point/range	°C 189	°F No data available
Melting point/range	°C 18.4	°F No data available
Flash point	°C 94	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	soluble	

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
dimethylsulfoxide	14500 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/ Potential Health effects

Eyes	Irritating to eyes.
Skin	Irritating to skin. Components of the product may be absorbed into the body through the skin.
Inhalation	May cause irritation of respiratory tract.
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — Eyes. Skin.

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

Proper shipping name Not classified as dangerous within the meaning of transport regulations

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
dimethylsulfoxide	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product contains the following HAPs:

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
dimethylsulfoxide	-	-	-	-	-

California Proposition 65
This product contains the following Proposition 65 chemicals:

WHMIS hazard class:
D2B Toxic materials

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet

invitrogen

Material Safety Data Sheet

Revision Date: 11-Feb-2008

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 18290015
Product name ELECTROMAX DH10B COMP CELLS

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
++1 716 774 6700

INVITROGEN CORPORATION NEW ZEALAND LIMITED
18 - 24 BOTHA ROAD
PENROSE
AUCKLAND 1006
NEW ZEALAND
0800-600-200

INVITROGEN AUSTRALIA PTY LIMITED
2A/14 LIONEL ROAD
MOUNT WAVERLY VIC 3149
AUSTRALIA
1-800-331-627

Emergency Number (Spills, Exposures): 301/431-8585 (24-hour)

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
Caution - substance not yet fully tested. To our knowledge, the hazards of this material have not been thoroughly investigated. We recommend handling all chemicals with caution

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

Form
Suspension

3. HAZARDS IDENTIFICATION

Principle Routes of Exposure/ Potential Health effects

Eyes	May cause eye irritation with susceptible persons.
Skin	May cause skin irritation in susceptible persons.
Inhalation	May cause irritation of respiratory tract.
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	Avoid contact with skin and eyes.
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Suspension

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	soluble	

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	May cause eye irritation with susceptible persons.
Skin	May cause skin irritation in susceptible persons.
Inhalation	May cause irritation of respiratory tract.
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous within the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	None
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
D2B Toxic materials

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

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End of Safety Data Sheet

MATERIAL SAFETY DATA SHEET

Revised 1/02/03
Replaces (None)
Printed 1/02/03

E. COLI STRAIN BL21-AI IN CCMB-80 BUFFER
INVITROGEN CORPORATION
MSDS ID: 500315

1. PRODUCT AND COMPANY INFORMATION

INVITROGEN CORPORATION
1600 FARADAY AVE.
CARLSBAD, CA 92008
760/603-7200

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716/774-6700

INVITROGEN CORPORATION
3 FOUNTAIN DR.
INCHINNAN BUSINESS PARK
PAISLEY, PA4 9RF
SCOTLAND
44-141 814-6100

INVITROGEN CORPORATION
P.O. BOX 12-502
PENROSE
AUCKLAND 1135
NEW ZEALAND
64-9-579-3024

INVITROGEN CORPORATION
2270 INDUSTRIAL ST.
BURLINGTON, ONT
CANADA L7P 1A1
905/335-2255

EMERGENCY NUMBER (SPILLS, EXPOSURES): 301/431-8585 (24 HOUR)
800/451-8346 (24 HOUR)
NON-EMERGENCY INFORMATION: 800/955-6288

Product Name:
E. COLI STRAIN BL21-AI IN CCMB-80 BUFFER

NOTE: If this product is a kit or is supplied with more than one material, please refer to the MSDS for each component for hazard information.

Product Use:
These products are for laboratory research use only and are not intended for human or animal diagnostics, therpeutic, or other clinical uses.

Synonyms:
Not available.

2. COMPOSITION, INFORMATION ON INGREDIENTS

The following list shows components of this product classified as hazardous based on physical properties and health effects:

Component	CAS No.	Percent
CALCIUM CHLORIDE	10043-52-4	0.5 - 1.5
GLYCEROL	56-81-5	7 - 13

3. HAZARDS IDENTIFICATION

********** EMERGENCY OVERVIEW **********
Warning!
Irritant.
Harmful if swallowed.
Harmful by inhalation.
****

Potential Health Effects:
Eye:
Contact with the eyes may cause moderate to severe eye injury. Eye contact may result in tearing and reddening, but not likely to permanently injure eye tissue. Temporary vision impairment (cloudy or blurred vision) is possible.

Skin:
Can cause severe irritation, defatting, and dermatitis. Irritation effects may last for hours or days but will not likely result in permanent damage.

Inhalation:
Can cause minor respiratory irritation, dizziness, weakness, fatigue, nausea, and headache.
Harmful! Can cause systemic damage (see "Target Organs).

Ingestion:
Severely irritating to mouth, throat, and stomach. Can cause abdominal discomfort, nausea, vomiting and diarrhea.
Harmful if swallowed. May cause systemic poisoning.

Chronic:
No data on cancer.

4. FIRST AID MEASURES

Eye:
Flush eyes with plenty of water for at least 20 minutes retracting eyelids often. Tilt the head to prevent chemical from transferring to the uncontaminated eye. Get immediate medical attention.

Skin:
Wash with soap and water. Remove contaminated clothing and launder. Get medical attention if irritation develops or persists.

Inhalation:
Remove to fresh air. If breathing is difficult, have a trained individual administer oxygen. If not breathing, give artificial respiration and have a trained individual administer oxygen. Get medical attention immediately.

4. FIRST AID MEASURES (CONT.)

Ingestion:
Severely irritating. Do not induce vomiting. Seek medical attention immediately. Drink 2 glasses of water or milk to dilute.

Note To Physician:
Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flashpoint Deg C:
Not available.

Upper Flammable Limit %:
Not available.

Lower Flammable Limit %:
Not available.

Autoignition Temperature Deg C:
Not available.

Extinguishing Media:
Use alcohol resistant foam, carbon dioxide, or dry chemical when fighting fires. Water or foam may cause frothing if liquid is burning but it still may be a useful extinguishing agent if carefully applied to the surface of the fire. Do Not direct a stream of water into the hot burning liquid. Use water spray/fog for cooling.

Firefighting Techniques/Equipment:
Do not enter fire area without proper protection including self-contained breathing apparatus and full protective equipment. Fight fire from a safe distance and a protected location due to the potential of hazardous vapors and decomposition products.

Hazardous Combustion Products:
Includes carbon dioxide, carbon monoxide, dense smoke.

6. ACCIDENTAL RELEASE MEASURES

Accidental releases may be subject to special reporting requirements and other regulatory mandates. Refer to Section 8 for personal protection equipment recommendations.

Spill Cleanup:
Exposure to the spilled material may be irritating or harmful. Follow personal protective equipment recommendations found in Section VIII of this MSDS. Additional precautions may be necessary based on special circumstances created by the spill including; the material spilled, the

6. ACCIDENTAL RELEASE MEASURES (CONT.)

quantity of the spill, the area in which the spill occurred. Also consider the expertise of employees in the area responding to the spill.
Ventilate the contaminated area.
Prevent the spread of any spill to minimize harm to human health and the environment if safe to do so. Wear complete and proper personal protective equipment following the recommendation of Section VIII at a minimum. Dike with suitable absorbent material like granulated clay. Gather and store in a sealed container pending a waste disposal evaluation.

7. HANDLING AND STORAGE

Storage of some materials is regulated by federal, state, and/or local laws.

Storage Pressure:
Ambient

Handling Procedures:
Harmful or irritating material. Avoid contacting and avoid breathing the material. Use only in a well ventilated area.
Keep closed or covered when not in use.

Storage Procedures:
Store in a cool dry ventilated location. Isolate from incompatible materials and conditions. Keep container(s) closed.
Suitable for most general chemical storage areas.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

Exposure Limits:

Component	OSHA PEL (ppm)	ACGIH TWA (ppm)
CALCIUM CHLORIDE	Not established.	Not established.
GLYCEROL	15	10 MG/M3

Engineering Controls:
Local exhaust ventilation or other engineering controls are normally required when handling or using this product to avoid overexposure.

Personal Protective Equipment:

Eye:
Mildly irritating but will not injure eye tissue.
An eye wash station must be available where this product is used.
Wear chemically resistant safety glasses with side shields when handling this product. Wear additional eye protection such as chemical splash goggles and/or face shield when the possibility exists for eye contact

8. EXPOSURE CONTROLS, PERSONAL PROTECTION (CONT.)

with splashing or spraying liquid, or airborne material. Do not wear contact lenses. Have an eye wash station available.

Skin:
Avoid skin contact by wearing chemically resistant gloves, an apron and other protective equipment depending upon conditions of use. Inspect gloves for chemical break-through and replace at regular intervals. Clean protective equipment regularly. Wash hands and other exposed areas with mild soap and water before eating, drinking, and when leaving work. Have a safety shower available.

Respiratory:
NIOSH approved air purifying respirator with dust/mist filter.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance/physical state:	Liquid solution / suspension
Odor:	No odor.
Boiling Point (C):	Not established.
Melting Point (C):	Not established.
Solubility in water:	Not established.
pH:	Not established.
Vapor Pressure:	Not established.
Vapor Density:	Not established.
Specific Gravity/Density:	Not established.
Octanol/water Partition Coeff:	Not established.
Volatiles:	Not established.
Evaporation Rate:	Not established.
Viscosity:	Not established.

10. STABILITY AND REACTIVITY

Stability:
Stable under normal conditions.

Conditions to Avoid:
Moisture. Metals. Strong oxidizing agents. Temperatures above flash point in combination with sparks, open flames, or other sources of ignition.

Hazardous Decomposition Products:
Hydrogen chloride. Chlorine containing gases. Carbon monoxide. Carbon dioxide.

Hazardous Polymerization:
Hazardous polymerization will not occur.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity:

Dermal/Skin:
Not determined.

Inhalation/Respiratory:
Not determined.

Oral/Ingestion:
Calcium Chloride: 1000 MG/KG
Glycerol: 12600 MG/KG

Target Organs: Heart. Kidneys.

Carcinogenicity:

NTP:
Not tested.

IARC:
Not listed.

OSHA:
Not regulated.

Other Toxicological Information

12. Ecological Information

Ecotoxicological Information: This material is soluble in water. It's adsorption to soil and sediment should not be significant.

Environmental Fate (Degradation, Transformation, and Persistence):
Bioconcentration is not expected to occur.
Biodegrades quickly.

13. DISPOSAL CONSIDERATIONS

Regulatory Information:
Not applicable.

Disposal Method:
Clean up and dispose of waste in accordance with all federal, state, and local environmental regulations.
Dispose of by incineration following Federal, State, Local, or Provincial regulations.

13. DISPOSAL CONSIDERATIONS (CONT.)

14. TRANSPORT INFORMATION

Proper Shipping Name: Not Determined.
Hazard Class:
Subsidiary Hazards:
ID Number:
Packing Group:

15. REGULATORY INFORMATION

UNITED STATES:

TSCA:
This product is solely for research and development purposes only and may not be used, processed or distributed for a commercial purpose. It may only be handled by technically qualified individuals.

Prop 65 Listed Chemicals: PROP 65 PERCENT
No Prop 65 Chemicals.

No 313 Chemicals

CANADA:

DSL/NDSL:
Not determined.

COMPONENT	WHMIS Classification
CALCIUM CHLORIDE	D2B
GLYCEROL	D2B

EUROPEAN UNION:

PRODUCT RISK PHRASES:
None assigned.

PRODUCT SAFETY PHRASES:
None assigned.

PRODUCT CLASSIFICATION:
None; Aucun; Geen; Keine; Nessuno; Ninguno/a

Component	EINECS Number
CALCIUM CHLORIDE	233-140-8

15. REGULATORY INFORMATION (CONT.)

GLYCEROL 200-289-5

16. OTHER INFORMATION

HMIS Rating 0-4:
FIRE: Not determined.
HEALTH: Not determined.
REACTIVITY: Not determined.

Abbreviations
N/A - Data is not applicable or not available
SARA - Superfund and Reauthorization Act
HMIS - Hazard Material Information System
WHMIS - Workplace Hazard Materials Information System
NTP - National Toxicology Program
OSHA - Occupational Health and Safety Administration
IARC - International Agency for Research on Cancer
PROP 65 - California Safe Drinking Water and Toxic Enforcement Act of 1986
EINECS - European Inventory of Existing Commercial Chemical Substances

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Public Health Agency of Canada | Agence de la santé publique du Canada

Canada

Adenovirus types 1, 2, 3, 4, 5 and 7 - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Adenovirus types 1, 2, 3, 4, 5 and 7***

SYNONYM OR CROSS REFERENCE: ARD, acute respiratory disease, pharyngoconjunctival fever

CHARACTERISTICS: *Adenoviridae*; non-enveloped, icosahedral virions, 70-90 nm diameter, doubled-stranded, linear DNA genome.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, tonsillitis, cough and conjunctivitis; common cause of nonstreptococcal exudative pharyngitis among children under 3 years; more severe diseases include laryngitis, croup, bronchiolitis, or severe pneumonia; a syndrome of pharyngitis and conjunctivitis (pharyngoconjunctival fever) is associated with adenovirus infection

EPIDEMIOLOGY: Worldwide; seasonal in temperate regions, with highest incidences in the fall, winter and early spring; in tropical areas, infections are common in the wet and colder weather; annual incidence is particularly high in children; adenovirus types 4 and 7 are common among military recruits (ARD)

HOST RANGE: Humans

INFECTIOUS DOSE: >150 plaque forming units when given intranasally

MODE OF TRANSMISSION: Directly by oral contact and droplet spread; indirectly by handkerchiefs, eating utensils and other articles freshly soiled with respiratory discharge of an infected person; outbreaks have been related to swimming pools; possible spread through the fecal-oral route

INCUBATION PERIOD: From 1-10 days

COMMUNICABILITY: Shortly prior to and for the duration of the active disease

SECTION III - DISSEMINATION

RESERVOIR: Humans

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available; cidofovir has shown promise in the treatment of adenoviral ocular infections.

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% sodium dodecyl sulfate

PHYSICAL INACTIVATION: Sensitive to heat>56°C; unusually stable to chemical or physical agents and adverse pH conditions

SURVIVAL OUTSIDE HOST: Resistance to chemical and physical agents allows for prolonged survival outside of the body. Adenovirus type 3 survived up to 10 days on paper under ambient conditions; adenovirus type 2 survived from 3-8 weeks on environmental surfaces at room temperature

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis

FIRST AID/TREATMENT: Mainly supportive therapy

IMMUNIZATION: Vaccine available for adenovirus types 4 and 7 (used for military recruits)

PROPHYLAXIS: None available

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: Ten cases documented up to 1988

SOURCES/SPECIMENS: Respiratory secretions

PRIMARY HAZARDS: Ingestion; droplet exposure of the mucous membrane

SPECIAL HAZARDS: Contact with feces from infected animals

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues

PROTECTIVE CLOTHING: Laboratory coat; gloves when skin contact with infectious materials is unavoidable

OTHER PRECAUTIONS: None

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing gently cover the spill with absorbent paper towel and apply 1% sodium hypochlorite starting at the perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization, incineration, chemical disinfection

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: November 1999

Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

Date Modified: 2001-01-23

Animal Viruses and Antisera

ATCC® Number:	**VR-5™** Order this Item	Price:	**$180.00**

Classification:	Adenoviridae, Mastadenovirus, Human adenovirus C
Agent:	Human adenovirus 5 deposited as Adenovirus type 5
Strain:	Adenoid 75
Original Source:	Spontaneously degenerating tissue culture of adenoid tissue from a 4-year-old girl with enlarged chronically infected tonsils, Washington DC, 1953
Depositors:	RJ Huebner, M Shaffer
Biosafety Level:	2
Shipped:	frozen
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Incubation :	**Atmosphere:** 5% CO2 in air recommended **Temperature:** 37.0°C **Duration:** 2-3 days; For best results cells should be 24 to 48 hours old and 80-90% confluent (not 100% confluent).
Effect :	Yes, in vitro effects: Cytopathic effects (cell rounding and sloughing)
Store at :	-70°C (or colder)
Comments :	Not inactivated by ether. Contains soluble group-specific CF antigen. Agglutinates rat RBC incompletely. Recognized as Reference Strain by Virus Subcommittee of the International Nomenclature Committee (Virology, 20: 613, 1963.)
Related Products:	Virus DNA: ATCC VR-5D
References :	24534: Huebner RJ, et al. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exp. Biol. Med. 84: 570-573, 1953. PubMed: 13134217 24544: Rowe WP, et al. Studies of the adenoidal-pharyngeal-conjunctival (APC) group of viruses. Am. J. Hyg. 61: 197-218, 1955. 24704: Hierholzer JC, et al. Antigenic relationships among the 47 human adenoviruses determined in reference horse antisera. Arch. Virol. 121: 179-197, 1991. PubMed: 1759904 92829: Chemical disinfectants and antiseptics --- Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine --- Test method and requirements (phase 2, step 1). London, UK:British Standards Institution;British Standard BS EN 14476:2005. 92837: Chemical disinfectants and antiseptics --- Application of European Standards for chemical disinfectants and antiseptics. London, UK:British Standards Institution;British Standard BS EN 14885:2006.

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Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Products

BioProducts

- Cell, microbial and molecular genomics products for the life sciences

BioServices

- Bio-materials management; basic repository to complex partnership-level services

BioStandards

- Biological Reference Material and Consensus Standards for the life science community

Return to Top

ThermoFisher SCIENTIFIC

Material Safety Data Sheet

Creation Date 23-Apr-2009 **Revision Date** 23-Apr-2009 **Revision Number** 1

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name Yeast Cultures

Cat No. Yeast Cultures

Synonyms No information available.

Recommended Use For research use only

Company
Thermo Fisher Scientific
Open Biosystem Products
601 Genone Way # 2100
Huntsville, AL 35806 United States
Tel: (303) 604-9499
Fax:(303) 604-9680

Emergency Telephone Number
Chemtrec US: (800) 424-9300
Chemtrec EU: (202) 483-7616

2. HAZARDS IDENTIFICATION

CAUTION!

Emergency Overview

May cause eye, skin, and respiratory tract irritation . Low hazard for usual industrial or commercial handling. The toxicological properties have not been fully investigated.

Appearance Light brown **Physical State** Liquid **odor** No information available

Target Organs None known.

Potential Health Effects

Acute Effects
Principle Routes of Exposure

Eyes	May cause irritation.
Skin	No known hazard in contact with skin.
Inhalation	May cause irritation of respiratory tract. Low hazard for usual industrial or commercial handling.
Ingestion	Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea. Low hazard for usual industrial or commercial handling.

Chronic Effects No information available.

See Section 11 for additional Toxicological information.

Aggravated Medical Conditions No information available..

3. COMPOSITION/INFORMATION ON INGREDIENTS

Haz/Non-haz

Component	CAS-No	Weight %
Water	7732-18-5	80
Peptones	73049-73-7	2
Yeast, ext.	8013-01-2	0.5
Sodium chloride	7647-14-5	0.5
Glycerin	56-81-5	15
Yeasts	RR-03602-2	5

4. FIRST AID MEASURES

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Obtain medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.

Inhalation Move to fresh air. If breathing is difficult, give oxygen. Get medical attention immediately if symptoms occur.

Ingestion Do not induce vomiting. Obtain medical attention.

Notes to Physician Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Flash Point Not applicable
Method No information available.

Autoignition Temperature No information available.

Explosion Limits
Upper No data available
Lower No data available

Suitable Extinguishing Media Substance is nonflammable; use agent most appropriate to extinguish surrounding fire..

Unsuitable Extinguishing Media No information available.

Hazardous Combustion Products No information available.

Sensitivity to mechanical impact No information available.
Sensitivity to static discharge No information available.

Specific Hazards Arising from the Chemical
Keep product and empty container away from heat and sources of ignition

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear

NFPA	Health 1	Flammability 0	Instability 0	Physical hazards N/A

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions Ensure adequate ventilation. Use personal protective equipment. Avoid contact with skin, eyes and clothing.

Environmental Precautions Should not be released into the environment.

Methods for Containment and Clean Up Soak up with inert absorbent material. Keep in suitable and closed containers for disposal.

7. HANDLING AND STORAGE

Handling Wear personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing.

Storage Long term. Keep container tightly closed. Keep at °C.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Engineering Measures Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location.

Exposure Guidelines

Component	ACGIH TLV	OSHA PEL	NIOSH IDLH
Glycerin	TWA: 10 mg/m³	(Vacated) TWA: 10 mg/m³ (Vacated) TWA: 5 mg/m³ TWA: 15 mg/m³ TWA: 5 mg/m³	

Component	Quebec	Mexico OEL (TWA)	Ontario TWAEV
Glycerin	TWA: 10 mg/m³	TWA: 10 mg/m³	TWA: 10 mg/m³

NIOSH IDLH: Immediately Dangerous to Life or Health

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure

Respiratory Protection Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State Liquid
Appearance Light brown

9. PHYSICAL AND CHEMICAL PROPERTIES

odor	No information available
Odor Threshold	No information available.
pH	Not applicable
Vapor Pressure	No information available.
Vapor Density	No information available.
Viscosity	No information available.
Boiling Point/Range	Not applicable
Melting Point/Range	No information available.
Decomposition temperature °C	No information available.
Flash Point	Not applicable
Evaporation Rate	No information available.
Specific Gravity	No information available.
Solubility	No information available.
log Pow	No data available

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Conditions to Avoid	Excess heat.
Incompatible Materials	None known
Hazardous Decomposition Products	None known
Hazardous Polymerization	Hazardous polymerization does not occur.
Hazardous Reactions .	None under normal processing..

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Sodium chloride	3 g/kg (Rat)	10 g/kg (Rabbit)	42 g/m^3 (Rat) 1 h
Glycerin	12600 mg/kg (Rat)	21900 mg/kg (Rat)	570 mg/m^3 (Rat) 1 h

Irritation	No information available.
Toxicologically Synergistic Products	No information available.

Chronic Toxicity

Carcinogenicity	There are no known carcinogenic chemicals in this product

Sensitization No information available.

Mutagenic Effects No information available.

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity No information available.

Other Adverse Effects The toxicological properties have not been fully investigated.

Endocrine Disruptor Information No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity

Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
Sodium chloride	Not listed	Pimephals prome: LC50: 7650 mg/L/96H	Not listed	EC50: 1000 mg/L/48H
Glycerin	Not listed	Not listed	Not listed	EC50 24 h >500 mg/L

Persistence and Degradability No information available

Bioaccumulation/ Accumulation No information available

Mobility

Component	log Pow
Water	-1.87
Glycerin	-1.76

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification

14. TRANSPORT INFORMATION

DOT Not regulated

TDG Not regulated

IATA Not regulated

IMDG/IMO Not regulated

14. TRANSPORT INFORMATION

15. REGULATORY INFORMATION

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	CHINA	KECL
Water	X	X	-	231-791-2	-		X	-	X	X	X
Peptones	X	X	-	-	-		X	-	X	X	KE-28131 X
Yeast, ext.	XU	X	-	232-387-9	-		X	-	X	X	KE-05-1355 X
Sodium chloride	X	X	-	231-598-3	-		X	X	X	X	KE-31387 X
Glycerin	X	X	-	200-289-5	-		X	X	X	X	KE-29297 X

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313
Not applicable

SARA 311/312 Hazardous Categorization

Acute Health Hazard	No
Chronic Health Hazard	No
Fire Hazard	No
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

Clean Water Act
Not applicable

Clean Air Act
Not applicable

OSHA
Not applicable

CERCLA
Not Applicable

California Proposition 65
This product does not contain any Proposition 65 chemicals.

State Right-to-Know

Component	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Glycerin	X	X	X	-	X

U.S. Department of Transportation

Reportable Quantity (RQ):	N
DOT Marine Pollutant	N
DOT Severe Marine Pollutant	N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade No information available

Canada

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

WHMIS Hazard Class
Non-controlled

16. OTHER INFORMATION

16. OTHER INFORMATION

Prepared By	Regulatory Affairs Thermo Fisher Scientific Tel: (412) 490-8929
Creation Date	23-Apr-2009
Print Date	23-Apr-2009
Revision Summary	"****", and red text indicates revision

Disclaimer

The information provided on this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.

End of MSDS

Cell line Info

Cell Biology

ATCC® Number: **CRL-1573™** Order this Item Price: **$256.00**

Designations:	293 [HEK-293]
Depositors:	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** embryonic kidney **Cell Type:** transformed with adenovirus 5 DNA
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications:	efficacy testing [92587] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) viruscide testing [92579]
Receptors:	vitronectin, expressed
Tumorigenic:	YES
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19
Cytogenetic Analysis:	

Related Links

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Cell Micrograph

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BioProducts

- Cell, microbial and molecular genomics products for the life sciences

BioServices

- Bio-materials management; basic repository to complex partnership-level services

BioStandards

- Biological Reference Material and Consensus Standards for the life science community

This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.

Age: fetus

Comments:

Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768]
The line is excellent for titrating human adenoviruses.
The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406]
The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.An inoculum of 2 X 10 (3) to 6 X 10 (3) viable cells/cm2 is recommended.
6. Incubate cultures at 37°C.6. Subculture when cell concentration is between 6 and 7 X 10(4) cells/cm2.

Subcultivation Ratio: 1:10 to 1:20 weekly.
Medium Renewal: Every 2 to 3 days

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003
derivative: ATCC CRL-10852
derivative: ATCC CRL-12006
derivative: ATCC CRL-12007
derivative: ATCC CRL-12013
derivative: ATCC CRL-12479
derivative: ATCC CRL-2029
derivative: ATCC CRL-2368
purified DNA:ATCC CRL-1573D

Cell Biology

ATCC® Number: **CCL-17™** Order this Item Price: **$318.00**

Additional information about this cell line

Designations:	KB
Depositors:	H Eagle
Biosafety Level:	2 [Cells contain human papilloma virus]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** HeLa contaminant
Cellular Products:	keratin
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 12,13.3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18
Isoenzymes:	G6PD, A
HeLa Markers:	Y
Comments:	This line was originally thought to be derived from an epidermal carcinoma of the mouth, but was subsequently found, based on isoenzyme analysis, HeLa marker chromosomes, and DNA fingerprinting, to have been established via HeLa cell contamination. The cells are positive for keratin by immunoperoxidase staining. KB cells have been reported to contain human papillomavirus 18 (HPV-18) sequences. NOTE: Cells of this line contain HeLa marker chromosomes, and were derived via HeLa contamination

Propagation:

Related Links

NCBI Entrez Search

2683.pdf

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Related Cell Culture Products

	ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. **Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:10 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium, 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor temperature
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020
References:	22214: Eagle H. Propagation in a fluid medium of a human epidermoid carcinoma, strain KB. Proc. Soc. Exp. Biol. Med. 89: 362-364, 1955. PubMed: 13254761 22766: Boshart M, et al. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J. 3: 1151-1157, 1984. PubMed: 6329740 26123: . . Science 133: 1559, 1961. 26124: Eagle H, Foley GE. Cytotoxicity in human cell cultures as a primary screen for the detection of anti-tumor agents. Cancer Res. 18: 1017-1025, 1958. PubMed: 13596943 26125: . . Proc. Soc. Exp. Biol. Med. 94: 661, 1957. 26126: Eagle H, et al. Viral susceptibility of a human carcinoma cell (strain KB). Proc. Soc. Exp. Biol. Med. 91: 361-364, 1956. PubMed: 13322936 32299: St. Geme JW, et al. Characterization of the genetic locus encoding Haemophilus influenzae type b surface fibrils. J. Bacteriol. 178: 6281-6287, 1996. PubMed: 8892830 32582: Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc. Natl. Acad. Sci. USA 93: 136-140, 1996. PubMed: 8552591 33028: Lee RJ, Huang L. Folate-targeted, anionic liposome-entrapped polylysine-condensed DNA for tumor cell-specific gene transfer. J. Biol. Chem. 271: 8481-8487, 1996. PubMed: 8626549

Return to Top

Cell Biology

ATCC® Number: **HTB-22™** Order this Item Price: **$272.00**

Designations:	MCF7
Depositors:	CM McGrath
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial
Cellular Products:	insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	estrogen receptor, expressed
Antigen Expression:	Blood Type O; Rh+
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 11 D16S539: 11,12 D5S818: 11,12 D7S820: 8,9 THO1: 6 TPOX: 9,12 vWA: 14,15
Cytogenetic Analysis:	modal number = 82; range = 66 to 87. The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.

Related Links

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Isoenzymes:	AK-1, 1 ES-D, 1-2 G6PD, B GLO-I, 1-2 PGM1, 1-2 PGM3, 1
Age:	69 years adult
Gender:	female
Ethnicity:	Caucasian
Comments:	The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.The cells express the WNT7B oncogene [PubMed: 8168088]. Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with anti-estrogens.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10% . **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

Subculturing:

Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.
Note: if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures.

1. Remove culture medium to a centrifuge tube.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	29 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020 purified DNA:ATCC HTB-22D purified RNA:ATCC HTB-22R 0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101 Cell culture tested DMSO:ATCC 4-X

Cell Biology

ATCC® Number:	**CRL-11268™**	Order this Item	Price:	**$272.00**

Designations:	293T/17 [HEK 293T/17]
Depositors:	Rockefeller Univ.
Biosafety Level:	2 [Cells contain Adeno and SV-40 viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** kidney
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	The line is available with the following restriction: 1. The cell line was deposited at the ATCCby Rockefeller University and is provided for research purposes only. Neither the cell line nor the products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as a service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the cells, or their products, must first be negotiated with Cell Genesys, 500 Forbes Boulevard, South San Francisco, CA 94080 Attn: Robert H. Tidwell; Senior Vice President, Corporate Development.
Antigen Expression:	SV40 T antigen [45408]
DNA Profile (STR):	Amelogenin: X CSF1PO: 11, 12 D13S317: 12, 14 D16S539: 9, 13 D5S818: 8, 9 D7S820: 11 THO1: 7, 9.3 TPOX: 11 vWA: 16, 18, 19
Age:	fetus

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BioProducts

Cell, microbial and molecular genomics products for the life sciences

BioServices

Bio-materials management; basic repository to complex partnership-level services

BioStandards

Biological Reference Material and Consensus Standards for the life science community

Comments:

The 293T/17 cell line is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted. 293T cells were cloned and the clones tested with the pBND and pZAP vectors to obtain a line capable of producing high titers of infectious retrovirus, 293T/17. These cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability.293T/17 cells were cotransfected with the pCRIPenv- and the pCRIPgag-2 vectors to obtain the ANJOU 65 (see ATCCCRL-11269) cell line.ANJOU 65 cells were cotransfected with the pCRIPgag-2 and pGPT2E vectors to obtain the BOSC 23 (see ATCCCRL-11270) ecotropic envelope-expression packaging cell line.ANJOU 65 cells were also cotransfected with the pCRIPAMgag vector along with a plasmid expressing the gpt resistance gene to obtain the Bing (see ATCCCRL-11554) amphotropic envelope-expression packaging cell line.

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO2), 5%

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Preservation:

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended
Medium Renewal: Every 2 to 3 days
Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020 derivative:ATCC CRL-11269
References:	45408: Sena-Esteves M, et al. Single-step conversion of cells to retrovirus vector producers with herpes simplex virus-Epstein-Barr virus hybrid amplicons. J. Virol. 73: 10426-10439, 1999. PubMed: 10559361 57446: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 5,952,225 dated Sep 14 1999 57447: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 6,329,199 dated Dec 11 2001 57448: Pear WS, et al. Production of High-Titer Helper-Free Retroviruses by Transient Transfection. Proc. Natl. Acad. Sci. USA 90: 8392-8396, 1993. PubMed: 7690960

Return to Top

Cell Biology

ATCC® Number: **CCL-121™** Order this Item Price: **$272.00**

Designations:	HT-1080
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Tissue:** connective tissue **Disease:** fibrosarcoma
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** July, 1972
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	Human poliovirus 1 RD-114 Feline Feline leukemia virus Vesicular stomatitis virus
Tumorigenic:	Yes
Oncogene:	ras +
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 12 D13S317: 12,14 D16S539: 9,12 D5S818: 11,13 D7S820: 9,10 THO1: 6 TPOX: 8 vWA: 14,19
Cytogenetic Analysis:	modal number = 46; range = 44 to 48. Pseudodiploidy was frequently noted. About 40% of the cells had rearranged karyotypes with an extra E-group chromosome and a group C chromosome, probably chromosome 11, was missing.
Isoenzymes:	G6PD, B
Age:	35 years
Gender:	male
Ethnicity:	Caucasian
Comments:	The cells contain an activated N-ras oncogene.

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Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C
Protocol:

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020

References:

22147: Chen TR, et al. Intercellular karyotypic similarity in near-diploid cell lines of human tumor origins. Cancer Genet. Cytogenet. 10: 351-362, 1983. PubMed: 6652615
23071: Geiser AG, et al. Suppression of tumorigenicity in human cell hybrids derived from cell lines expressing different activated ras oncogenes. Cancer Res. 49: 1572-1577, 1989. PubMed: 2647289
23393: Rasheed S, et al. Characterization of a newly derived human sarcoma cell line (HT-1080). Cancer 33: 1027-1033, 1974. PubMed: 4132053
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Return to Top

Cell Biology

ATCC® Number:	**CCL-185™**	Order this Item	Price:	**$256.00**

Designations:	A549
Depositors:	M Lieber
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** lung **Disease:** carcinoma
Cellular Products:	keratin
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** 1972
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,12 D13S317: 11 D16S539: 11,12 D5S818: 11 D7S820: 8,11 THO1: 8,9.3 TPOX: 8,11 vWA: 14
Cytogenetic Analysis:	This is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Cells with 64 (22%), 65, and 67 chromosome counts also occurred at relatively high frequencies; the rate with higher ploidies was low at 0.4%. There were 6 markers present in single copies in all cells. They include der(6)t(1;6) (q11;q27); ?del(6) (p23); del (11) (q21), del(2) (q11), M4 and M5. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40% of 50 cells analyzed. Chromosomes N2 and N6 had single copies per cell; and N12 and N17 usually had 4 copies.
Isoenzymes:	G6PD, B
Age:	58 years

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Gender:	male
Ethnicity:	Caucasian
Comments:	This line was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male. [23218] Further studies by M. Lieber, et al. revealed that A549 cells could synthesize lecithin with a high percentage of desaturated fatty acids utilizing the cytidine diphosphocholine pathway. [58030] The cells are positive for keratin by immunoperoxidase staining.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Protocol:**
Subculturing:	1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. Cultures can be established between 2 X 10(3) and 1 X 10(4) viable cells/cm2. Do not exceed 7 X 10(4) cels/cm2. 6. Incubate cultures at 37°C.
Preservation:	**Interval:** Maintain cultures at a cell concentration between 6 X 10(3) and 6 X 10(4) cell/cm2. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:8 is recommended **Medium Renewal:** 2 to 3 times per week **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	about 22 hours

Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004 recommended serum:ATCC 30-2020

References:

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32347: Horikami SM, et al. The Sendai virus V protein interacts with the NP protein to regulate viral genome RNA replication. Virology 222: 383-390, 1996. PubMed: 8806522
32351: Huang S, et al. Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells. J. Virol. 70: 4502-4508, 1996. PubMed: 8676475
32373: Goodrum FD, et al. Adenovirus early region 4 34-kilodalton protein directs the nuclear localization of the early region 1B 55-kilodalton protein in primate cells. J. Virol. 70: 6323-6335, 1996. PubMed: 8709260
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32514: Bartz SR, et al. Human immunodeficiency virus type 1 cell cycle control: Vpr is cytostatic and mediates G2 accumulation by a mechanism which differs from DNA damage checkpoint control. J. Virol. 70: 2324-2331, 1996. PubMed: 8642659
32722: Garofalo R, et al. Transcriptional activation of the interleukin-8 gene by respiratory syncytial virus infection in alveolar epithelial cells: nuclear translocation of the RelA transcription factor as a mechanism producing airway mucosal inflammation. J. Virol. 70: 8773-8781, 1996. PubMed: 8971006

32758: Jamaluddin M, et al. Inducible translational regulation of the NF-IL6 transcription factor by respiratory syncytial virus infection in pulmonary epithelial cells. J. Virol. 70: 1554-1563, 1996. PubMed: 8627674
33091: Lewis JA, et al. Inhibition of mitochondrial function by interferon. J. Biol. Chem. 271: 13184-13190, 1996. PubMed: 8662694
58030: Lieber M, et al. A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. Int. J. Cancer 17: 62-70, 1976. PubMed: 175022

Return to Top

Cell Biology

ATCC® Number:	**CCL-2™** Order this Item	Price:	**$256.00**

Designations:	HeLa
Depositors:	WF Scherer
Biosafety Level:	2 [Cells contain human papilloma virus]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** cervix **Disease:** adenocarcinoma **Cell Type:** epithelial
Cellular Products:	keratin Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host ([21491] Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) screening for Escherichia coli strains with invasive potential [21447] [21491]
Virus Susceptibility:	Human adenovirus 3 Encephalomyocarditis virus Human poliovirus 1 Human poliovirus 2 Human poliovirus 3
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 12,13.3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18

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Cytogenetic Analysis:	Modal number = 82; range = 70 to 164. There is a small telocentric chromosome in 98% of the cells. 100% aneuploidy in 1385 cells examined. Four typical HeLa marker chromosomes have been reported in the literature.HeLa Marker Chromosomes: One copy of Ml, one copy of M2, four-five copies of M3, and two copies of M4 as revealed by G-banding patterns. M1 is a rearranged long arm and centromere of chromosome 1 and the long arm of chromosome 3. M2 is a combination of short arm of chromosome 3 and long arm of chromosome 5. M3 is an isochromosome of the short arm of chromosome 5. M4 consists of the long arm of chromosome 11 and an arm of chromosome 19.
Isoenzymes:	G6PD, A
Age:	31 years adult
Gender:	female
Ethnicity:	Black
HeLa Markers:	Y
Comments:	The cells are positive for keratin by immunoperoxidase staining. HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences. P53 expression was reported to be low, and normal levels of pRB (retinoblastoma suppressor) were found.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Preservation:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020 Also see CCL-2.1 derivative:ATCC CCL-2.2 derivative:ATCC CCL-2.3

Cell Biology

ATCC® Number: **HTB-96™** Order this Item Price: **$256.00**

Designations:	U-2 OS
Depositors:	Hellstrom
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** bone **Disease:** osteosarcoma
Cellular Products:	osteosacoma derived growth factor (ODGF)
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	insulin-like growth factor I (IGF-I); insulin-like growth factor II (IGF II)
Antigen Expression:	Blood Type A; Rh+; HLA A2, Aw30, B12, Bw35, B40(+/-)
DNA Profile (STR):	Amelogenin: X CSF1PO: 13 D13S317: 13 D16S539: 11,12 D5S818: 11 D7S820: 11,12 THO1: 6,9.3 TPOX: 11,12 vWA: 14,18
Cytogenetic Analysis:	Cell line U-2 OS is chromosomally highly altered, with chromosome counts in the hypertriploid range. We did not find the hypodiploid cell population described by J. Ponten, et al., Instead, most of the population has slightly higher counts than first described. Very few normal chromosomes are present, but a high number of stable marker chromosomes are identified., Different chromosomal rearrangements involving the same chromosomes (N1, N7, N9, and N11 particularly), are seen. Twenty-two markers are found including: t(9qter---->9q21::1p36--->1p::?), 7p+, iso(17q), t(15q;?), 4q+, del(3)(q21), 5q(aberrant) and others. [22509]

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Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 PGM1, 2 PGM3, 1
Age:	15 years
Gender:	female
Ethnicity:	Caucasian
Comments:	J. Ponten and E. Saksela derived this line (originally 2T) in 1964 from a moderately differentiated sarcoma of the tibia of a 15 year old girl. Viruses were not detected during co-cultivation with WI-38 cells or in CF tests against SV40, RSV or adenoviruses. Mycoplasma contamination was detected and eliminated in 1972.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated McCoy's 5a Medium Modified, Catalog No. 30-2007. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.
Preservation:	Culture medium, 95%; DMSO, 5%
Related Products:	recommended serum:ATCC 30-2020

References:

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22509: Ponten J, Saksela E. Two established in vitro cell lines from human mesenchymal tumours. Int. J. Cancer 2: 434-447, 1967. PubMed: 6081590
23011: Raile K, et al. Human osteosarcoma (U-2 OS) cells express both insulin-like growth factor-I (IGF-I) receptors and insulin-like growth factor-II/mannose-6- phosphate (IGF-II/M6P) receptors and synthesize IGF-II: autocrine growth stimulation by IGF-II via the IGF-I receptor. J. Cell. Physiol. 159: 531-541, 1994. PubMed: 8188767
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Return to Top

Cell Biology

ATCC® Number:	**CCL-186™**	Order this Item	Price:	**$256.00**

Designations:	IMR-90
Depositors:	WW Nichols
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	fibroblast
Source:	**Organ:** lung **Disease:** normal **Cell Type:** fibroblast
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza)
Virus Susceptibility:	Human poliovirus 1 Human poliovirus 2 Varicella-Zoster Herpes simplex virus 1 Herpes simplex virus 2 Human poliovirus 3 Vaccinia virus Human herpesvirus 5 Vesicular stomatitis virus
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,13 D13S317: 11,13 D16S539: 10,13 D5S818: 12,13 D7S820: 9,12 THO1: 8,9.3 TPOX: 8,9 vWA: 16,19
Cytogenetic Analysis:	normal human female; diploid; stable
Isoenzymes:	G6PD, B
Age:	16 weeks gestation
Gender:	female
Ethnicity:	Caucasian

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Comments:	The human diploid fibroblast strain IMR-90 was derived by W.W. Nichols and associates from the lungs of a 16-week female fetus. [22381] The division potential, viral susceptibilities and other properties have been thoroughly studied such that the line may be considered as an alternate for WI-38 and other standard human lung cell strains. The cells have been reported to be capable of attaining 58 population doublings before the onset of senescence. [22381]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Protocol:**
Subculturing:	1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C.
Preservation:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:8 is recommended **Medium Renewal:** Every 3 to 4 days **Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020
References:	

22381: Nichols WW, et al. Characterization of a new human diploid cell strain, IMR-90. Science 196: 60-63, 1977. PubMed: 841339
32932: Dolganov GM, et al. Human Rad50 is physically associated with human Mre11: identification of a conserved multiprotein complex implicated in recombinational DNA repair. Mol. Cell. Biol. 16: 4832-4841, 1996. PubMed: 8756642
33041: Ostlund RE Jr., et al. A sterospecific myo-inositol/D-chiro-inositol transporter in HepG2 liver cells. J. Biol. Chem. 271: 10073-10078, 1996. PubMed: 8626564

Return to Top

Cell Biology

ATCC® Number:	**CCL-75™**	Order this Item	Price:	**$272.00**

Designations:	WI-38
Depositors:	L Hayflick
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	fibroblast
Source:	**Organ:** lung **Disease:** normal **Cell Type:** fibroblast
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	testing [92346] [92389] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) viruscide testing
Virus Susceptibility:	Vesicular stomatitis, Glasgow (Indiana) Herpes simplex virus Pseudorabies virus Human poliovirus 1
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,12 D13S317: 11 D16S539: 11,12 D5S818: 10 D7S820: 9,11 THO1: 8,9.3 TPOX: 8 vWA: 19,20
Cytogenetic Analysis:	normal diploid
Isoenzymes:	G6PD, B
Age:	3 months gestation fetus
Gender:	female
Ethnicity:	Caucasian

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	The WI-38 human diploid cell line was derived by Leonard Hayflick from normal embryonic (3 months gestation) lung tissue. [22194] WI-38 cells have a finite lifetime of 50 plus or minus 10 population doublings with a doubling time of 24 hours. [22197]
Comments:	This line was the first human diploid cell line to be used in human vaccine preparation. [26220] The 8th passage ampule from which this freeze was derived was found to contain a bacterial contaminant (a micrococcus). The cell line was subsequently cured by several passages in the presence of antibiotics. Growth of the cells is enhanced by addition of tumor necrosis factor alpha (TNF alpha) to the medium.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	Trypsin and medium should be prewarmed to 37C and the pH should be adjusted to 7.5 before use. Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	growth medium, 95%; DMSO, 5%
Doubling Time:	24 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020

References:

21405: Sugarman BJ, et al. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. Science 230: 943-945, 1985. PubMed: 3933111
22194: Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp. Cell Res. 25: 585-621, 1961. PubMed: 13905659
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26223: . First International Conference on Vaccines against Viral and Rickettsial Diseases of Man. Pan Am. Health Organ. Off. Doc. 147: 581, 1967.
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92346: Biological evaluation of medical devices. Part 5: Tests for in vitro cytotoxicity. Sydney, NSW, Australia:Standards Australia;Standards Australia AS ISO 10993.5-2002.
92389: Biological evaluation of medical devices--Part 5: Tests for in vitro cytotoxicity. Geneva (Switzerland):International Organization for Standardization/ANSI;ISO ISO 10993-5:1999.

Return to Top

Cell Biology

ATCC® Number:	**HTB-85™**	Order this Item	Price:	**$269.00**

Designations:	Saos-2
Depositors:	J Fogh, G Trempe
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** bone **Disease:** osteosarcoma
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	The cells are distributed for research purposes only. The Memorial Sloan-Kettering Cancer Center releases the line subject to the following: 1.) The cells or their products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of these cells must first be negotiated with The Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	epidermal growth factor (EGF); transforming growth factor beta (type 1 and type 2)
Tumorigenic:	No
Antigen Expression:	Blood Type B, Rh+; HLA A2, A3, Bw16, Bw47
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 12,13 D16S539: 12,13 D5S818: 12 D7S820: 8,10 THO1: 6,9 TPOX: 8 vWA: 18

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Cytogenetic Analysis:	The stemline chromosome number is hypotriploid with the modal number of 56 chromosomes per cell and the 2S component occurring at 13.2%. Over two-thirds of the chromosome complement consisted of structurally rearranged chromosomes., Most marker chromosomes had complex rearrangements. The origin of the segments composing these markers could not be identified. Of the identifiable markers, 6p+/q+, 7p+, 11p+, and 12p+ occasionally were present at 2 copies per cell., The Y chromosome was not detected in the QM stained preparation.
Isoenzymes:	AK-1, 1 ES-D, 2 G6PD, B GLO-I, 2 Me-2, 1 PGM1, 1-2 PGM3, 1-2
Age:	11 years
Gender:	female
Ethnicity:	Caucasian
Comments:	This is one of an extensive series of human tumor lines isolated and characterized by J. Fogh and G. Trempe. The patient was treated with RTG, methotrexate, adriamycin, vincristine, cytoxan, and aramycin-C.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated McCoy's 5a Medium Modified , Catalog No. 30-2007. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 15%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended **Medium Renewal:** 1 to 2 times per week Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.
Preservation:	Culture medium, 95%; DMSO, 5%
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2007 recommended serum:ATCC 30-2020

References:

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Return to Top

Cell Biology

ATCC® Number:	CRL-1550™ Order this Item	Price:	$264.00
Designations:	Ca Ski		
Depositors:	RA Pattillo		
Biosafety Level:	2 [Cells contain human papilloma virus]		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial		
Source:	**Organ:** cervix **Disease:** epidermoid carcinoma **Derived from metastatic site:** small intestine		
Cellular Products:	beta subunit of human chorionic gonadotropin (hCG); tumor associated antigen		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 8,12 D16S539: 11,12 D5S818: 13 D7S820: 8,11 THO1: 7 TPOX: 8 vWA: 17		
Isoenzymes:	G6PD, B		
Age:	40 years adult		
Gender:	female		
Ethnicity:	Caucasian		
Comments:	The line was established from cells from a metastasis in the small bowel mesentery. The cells are reported to contain an integrated human papillomavirus type 16 genome (HPV-16, about 600 copies per cell) as well as sequences related to HPV-18.		
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%		
Subculturing:	**Protocol:** 1.Remove and discard culture medium. 2.Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3.Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4.Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5.Add appropriate aliquots of the cell suspension to new culture vessels. 6.Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:8 is recommended **Medium Renewal:** Every 2 to 3 days		
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase		
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020 Also see	References:	

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Return to Top

Cell Biology

ATCC® Number:	**HB-8065™**	Order this Item	Price:	**$272.00**

Designations:	Hep G2
Depositors:	Wistar Institute
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** liver **Disease:** hepatocellular carcinoma
Cellular Products:	alpha-fetoprotein (alpha fetoprotein); albumin; alpha2 macroglobulin (alpha-2-macroglobulin); alpha1 antitrypsin (alpha-1-antitrypsin); transferrin; alpha1 antichymotrypsin; (alpha-1-antichymotrypsin); haptoglobin; ceruloplasmin; plasminogen; [3525] complement (C4); C3 activator; fibrinogen; alpha1 acid glycoprotein (alpha-1 acid glycoprotein); alpha2 HS glycoprotein (alpha-2-HS-glycoprotein); beta lipoprotein (beta-lipoprotein); retinol binding protein (retinol-binding protein) [3525]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	insulin; insulin-like growth factor II (IGF II) [22446]
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 9,13 D16S539: 12,13 D5S818: 11,12 D7S820: 10 F13A01: 5,7 F13B: 6,10 FESFPS: 11 LPL: 10,11 THO1: 9 TPOX: 8,9 vWA: 17

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Cytogenetic Analysis:	modal number = 55 (range = 50 to 60); has a rearranged chromosome 1 [3525]
Age:	15 years adolescent
Gender:	male
Ethnicity:	Caucasian
Comments:	The cells express 3-hydroxy-3-methylglutaryl-CoA reductase and hepatic triglyceride lipase activities. [23557] The cells demonstrate decreased expression of apoA-I mRNA and increased expression of catalase mRNA in response to gramoxone (oxidative stress). [26594] There is no evidence of a Hepatitis B virus genome in this cell line. [1205] [22909]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:6 is recommended **Medium Renewal:** Twice per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020 derivative:ATCC CRL-10741 derivative:ATCC CRL-11997 purified DNA:ATCC HB-8065D

References:

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Return to Top

Cell Biology

ATCC® Number:	HTB-31™ Order this Item
Designations:	C-33 A
Depositors:	N Auersperg
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** cervix **Disease:** carcinoma
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Tumorigenic:	Yes
Oncogene:	p53 +; pRB +
DNA Profile (STR):	Amelogenin: X CSF1PO: 12 D13S317: 13 D16S539: 13,14 D5S818: 11,12 D7S820: 10 THO1: 7,8 TPOX: 9 vWA: 18,20
Cytogenetic Analysis:	This a pseudodiploid human cell line with the modal chromosome number of 46, occurring in 70% of cells examined. Polyploid cells occurred at 8.6%. Seven marker chromosomes were consistently detected per pseudodiploid cell. They are: t(1q17q), t(1p21q), del(18) (q21.3), der(1)t(1;17) (p16;q21.3) and three others. Several other markers were also found but they occurred only once in 15 metaphases analyzed. Neither DMs nor HSRs were detected. Structurally normal N1 was absent. Generally there are two X chromosomes in each cell.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 Me-2, 2 PGM1, 1 PGM3, 1
Age:	66 years adult
Gender:	female
Ethnicity:	Caucasian
Comments:	The C-33 A cell line is one of a series of lines (see also ATCCCRL-1594 and ATCCCRL-1595) derived by N. Auersperg from cervical cancer biopsies. The line exhibited a hypodiploid karyotype initially, and an epithelial morphology. Karyological instability was observed with continued passage. The retinoblastoma protein (pRB) is present but abnormal in size. Expression of p53 is elevated, and there is a point mutation at codon 273 resulting in a Arg -> Cys substitution. The cells are negative for human papillomavirus DNA and RNA.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%
Subculturing:	**Protocol:** Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:8 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Culture medium, 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase

Price: **$273.00**

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Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020 Also see Certified Reference Material	References:	22515: . . J. Natl. Cancer Inst. 32: 135-148, 1964. 23180: Yee C, et al. Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. Am. J. Pathol. 119: 361-366, 1985. PubMed: 2990217 23324: Scheffner M, et al. The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. Proc. Natl. Acad. Sci. USA 88: 5523-5527, 1991. PubMed: 1648218 29988: Hendricks DT, et al. FHIT gene expression in human ovarian, endometrial, and cervical cancer cell lines. Cancer Res. 57: 2112-2115, 1997. PubMed: 9187105 32507: Kovelman R, et al. Enhanced transcriptional activation by E2 proteins from the oncogenic human papillomaviruses. J. Virol. 70: 7549-7560, 1996. PubMed: 8892874

Return to Top

Cell Biology

ATCC® Number: **CRL-1658™** Order this Item Price: **$256.00**

Designations:	NIH/3T3
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	fibroblast 
Source:	**Organ:** embryo **Strain:** NIH/Swiss **Cell Type:** fibroblast fibroblast;
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	Murine leukemia virus
Age:	embryo
Comments:	The NIH/3T3 is highly sensitive to sarcoma virus focus formation and leukemia virus propagation and has proven to be very useful in DNA transfection studies [PubMed ID: 222457]. Tested and found negative for ectromelia virus (mousepox).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: bovine calf serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Growth Conditions:** The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum. The calf serum initially employed and found to be satisfactory was from the Colorado Serum Co. Denver.

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Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37C. . DO NOT ALLOW THE CELLS TO BECOME CONFLUENT! Subculture at least twice per week at 80% confluence or less. **Subcultivation Ratio:** Inoculate 3 to 5 X 10(3) cells/cm2 **Medium Renewal:** Twice per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

References:

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Return to Top

Cell Biology

ATCC® Number:	**SCRC-1008™**	Order this Item	Price:	**$129.00**

Designations:	MEF (C57BL/6) [MEF-BL/6-1]
Depositors:	ATCC
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	fibroblast
Source:	**Strain:** C57BL/6 **Organ:** embryo, whole **Cell Type:** fibroblast
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	United States **Isolation date:** January 22, 2003
Age:	14 days gestation embryo
Gender:	male and female mixed
Comments:	The cell line was established by ATCCin 2003 from dissociated C57BL/6 mouse embryos. The cells can be used as a feeder layer to support the growth of embryonic stem (ES) cells and for the maintenance of ES cells in the undifferentiated state. The growth of these cells must be arrested before they can be used as a feeder layer. ATCChas successfully treated the cells with mitomycin C for use as a feeder layer. If the MEFs are being used as a feeder layer for ES cells, it is not recommended to use them past passage no. 6 (P6).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: • fetal bovine serum to a final concentration of 15% **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

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Subculturing:

Protocol: Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 1X PBS (Ca+2/Mg+2-free, ATCCcat# SCRR-2201) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of 0.25% (w/v) Trypsin/0.53mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Preservation:

Subcultivation Ratio: 1:5 to 1:8

Freeze medium: Complete growth medium, supplemented with an additional 40% FBS and 10% DMSO(v/v)

Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020
derivative:ATCC SCRC-1008.1
derivative:ATCC SCRC-1008.2

Return to Top

Cell Biology

ATCC® Number:	**CCL-70™**	Order this Item	Price:	**$289.00**

Designations:	CV-1
Depositors:	FC Jensen
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Cercopithecus aethiops*
Morphology:	fibroblast
Source:	**Organ:** kidney **Disease:** normal
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	efficacy testing [92587] transfection host (Roche FuGENE® Transfection Reagents) viruscide testing [92579] [92582]
Cytogenetic Analysis:	This is a pseudodiploid, male African green monkey cell line. The modal chromosome number was 60, occurring in 48% of cells, and the rate of polyploidy was at 4.4%. Only a few markers were found., Of these M1, a probable deleted N11, was found in all cells examined; M3 of unknown origin was in some cells; and the remaining 2 to 3 others of unknown origins were found only once., N11 was uniformly single copied, and N16 was also single copied in most cells. Both X and Y chromosomes were also detected in every cell.
Age:	141 days
Gender:	male
Comments:	Suitable host for transfection, especially by SV40 vectors. The CV-1 cell line was derived from the kidney of a male adult African green monkey by F.C. Jensen, et al. in March, 1964 for use in Rous sarcoma virus transformation studies. [22138] Derived from the kidney of male adult African green monkey.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C

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Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:3 is recommended **Medium Renewal:** 2 to 3 times per week Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.
Preservation:	Culture medium, 95%; DMSO, 5%
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020 derivative:ATCC CRL-1650 derivative:ATCC CRL-1651

References:

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92582: Standard Test Method for Evaluation of Handwashing Formulation for Virus-Eliminating Activity using the Entire Hand. West Conshohocken, PA:ASTM International;ASTM Standard Test Method E 2011-99.
92587: Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides. West Conshohocken, PA:ASTM International;ASTM Standard Test Method E 2197-02.

Return to Top

Cell Biology

ATCC® Number:	**CCL-81™**	Order this Item	Price:	**$256.00**

Designations:	Vero
Depositors:	W Hann, JS Rhim
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Cercopithecus aethiops*
Morphology:	epithelial 
Source:	**Organ:** kidney **Disease:** normal
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** March 27, 1962
Applications:	detection of verotoxin [21447] efficacy testing [92579] [92587] malaria biology media testing [11019] mycoplasma testing [92577] substrate [92447] testing [34219] [92309] [92319] [92320] [92321] [92322] [92324] [92346] [92389] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) detection of virus in ground beef [34219]
Virus Resistance:	Stratford; Apeu; Caraparu; Madrid; Nepuyo; Ossa
Cytogenetic Analysis:	This is a cell line with the hypodiploid chromosome count. The modal chromosome number was 58 occurring in 66% of cells. In most cells, over 50% of the chromosomes in each cell complement belonged to structurally altered marker chromosomes. Normal A3, A4, B4, and B5 were absent; B2, B3 and B7 were occasionally paired; and B9, C1 and C5 were mostly paired. The rate of cells with higher ploidies was 1.7%. Other chromosomes were mostly present in single copy.
Age:	adult
Comments:	

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Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

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Product Information Sheet

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- Biological Reference Material and Consensus Standards for the life science community

The Vero cell line was initiated from the kidney of a normal adult African green monkey on March 27, 1962, by Y. Yasumura and Y. Kawakita at the Chiba University in Chiba, Japan. [21447]
The cell line was brought to the Laboratory of Tropical Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health in the 93rd passage from Chiba University by B. Simizu on June 15, 1964.

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
derivative:ATCC CRL-1587

Cell Biology

ATCC® Number:	**CRL-1650™**	Order this Item	Price:	**$275.00**

Designations:	COS-1
Depositors:	Y Gluzman
Biosafety Level:	2 [Cells Contain PAPOVAVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Cercopithecus aethiops*
Morphology:	fibroblast
Source:	**Organ:** kidney **Cell Type:** SV40 transformed
Cellular Products:	T antigen
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Comments:	This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region.The line was derived from the CV-1 cell line (ATCC® CCL-70) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen. The cells contain a single integrated copy of the complete early region of the SV40 genome.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

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Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

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Protocol:

Subculturing:

1. Remove and discard medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually 5 to 10 min).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detatch. Cells that are difficult to detatch may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37C.
   Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended
   Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor temperature

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020
parental cell line:ATCC CCL-70
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:

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Return to Top

Cell Biology

ATCC® Number: **CRL-1651™** Order this Item Price: **$269.00**

Designations:	COS-7
Depositors:	Y Gluzman
Biosafety Level:	2 [Cells Contain SV-40 viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Cercopithecus aethiops*
Morphology:	fibroblast 
Source:	**Organ:** kidney **Cell Type:** SV40 transformed
Cellular Products:	T antigen
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Comments:	This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region.The line was derived from the CV-1 cell line (ATCC® CCL-70?) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

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Biological Reference Material and Consensus Standards for the life science community

Protocol:

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020
parental cell line:ATCC CCL-70
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

Cell Biology

ATCC® Number:	**CRL-1711™**	Order this Item	Price:	**$275.00**

Designations:	Sf9
Depositors:	G Smith, C Cherry, MD Summers
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	mixed: adherent/suspension
Organism:	Spodoptera frugiperda (fall armyworm)
Morphology:	epithelial 
Source:	**Organ:** ovary
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** 1983
Applications:	transfection host (Roche FuGENE® Transfection Reagents technology from amaxa)
Virus Susceptibility:	Nuclear polyhedrosis virus, Autographa californica St. Louis encephalitis virus
Age:	pupa
Gender:	female
Comments:	This line can be used to replicate baculovirus expression vectors. It is important to use the medium described below.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is Grace's Insect Medium Supplemented (GIBCO/Invitrogen Cat. No. 11605-094 or equivalent). To make the complete growth medium, add the following components to the base medium: heat-inactivated insect cell culture tested fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 100% **Temperature:** 28.0°C **Growth Conditions:** The recommended media are formulated for use without CO2. Omission of the yeastolate or lactalbumin hydrolysate will lead to poor performance by this line.

Subculturing:

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Technical Support

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Protocol: Gently resuspend cells in the spent culture medium by pipetting across the monolayer or by hitting the flask against the palm of your hand (the latter is only preferable when working with larger flasks).
Subcultivation Ratio: A subcultivation ratio of 1:5 or greater is recommended
Medium Renewal: Every 2 to 3 days

Preservation:

Freeze medium: culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

References:

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Return to Top

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 11497013
Product name Sf21 Cells (SFM Adapted) 10e7 cells

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
dimethylsulfoxide	67-68-5	7-13

The product contains no substances which at their given concentration, are considered to be hazardous to health.

3. HAZARDS IDENTIFICATION

Emergency Overview
Components of the product may be absorbed into the body through the skin

3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	Mild eye irritation.
Skin	moderate skin irritation. Components of the product may be absorbed into the body through the skin.
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	1
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
dimethylsulfoxide	-	-	-	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory Protection In case of insufficient ventilation wear suitable respiratory equipment

Hand protection Impervious butyl rubber gloves. Nitrile gloves are not recommended. Some brands of Nitrile gloves have breakthrough times of five minutes.. Nitrile gloves are not recomended. Some brands of Nitrile gloves have breakthrough times of five minutes.

.

Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing.
Hygiene measures Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	soluble	

10. STABILITY AND REACTIVITY

Stability Stable.
Materials to avoid No information available
Hazardous decomposition products No information available
Polymerization Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
dimethylsulfoxide	14500 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/ Potential Health effects

Eyes	Mild eye irritation.
Skin	moderate skin irritation. Components of the product may be absorbed into the body through the skin.
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects **(Long Term Effects)**

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
dimethylsulfoxide	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
dimethylsulfoxide	-	-	-	-	-

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
D2B Toxic materials

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

For research use only

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

End of Safety Data Sheet

invitrogen™

Material Safety Data Sheet

Revision Date: 01-May-2007

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 12290010
Product name pcDNA™3.1/nV5-DEST

Section 4.0

Contact manufacturer
INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health.

Form
Solid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures — Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Solid

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product contains the following HAPs:

U.S. State Regulations

California Proposition 65
This product contains the following Proposition 65 chemicals:

WHMIS hazard class:
Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet

Vector Information

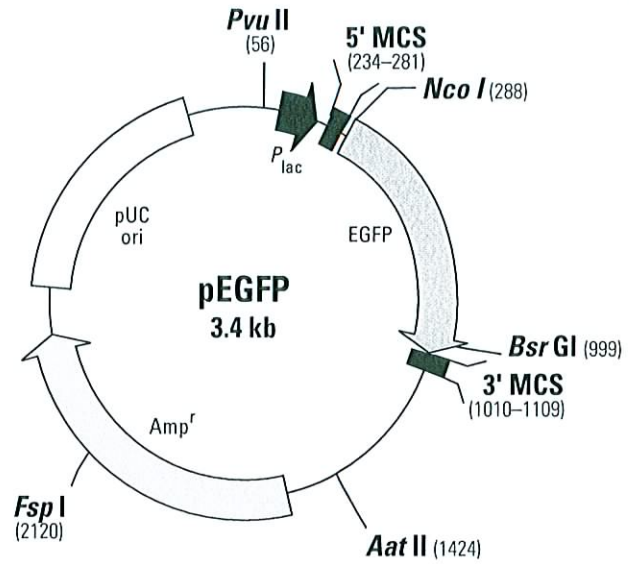
Specific EGFP Monoclonal Antibody for Westerns, IP and IC

Visit our website for more details! click here...

pEGFP Vector Information

PT3078-5

Catalog #6077-1



5' MCS

lacZ →

230 240 250 260 270 280

ATG ACC ATG ATT ACG CCA AGC TTG CAT GCC TGC AGG TCG ACT CTA GAG GAT CCC CGG GTA CCG GTC GCC ACC ATG GTG → EGFP

Hind III Sph I Pst I Acc I Sal I Hinc II Xba I Bam HI Xma I Sma I Kpn I Asp718 I Age I Nco I

3' MCS

EGFP STOP 1010

TAA AGCGGCCGCGACTCTAGAATTCCAACTGAGCGCCGGTCGCTACCATTACCAACTTGTCTGGTGTCAAAAATAATAGGCCT

Not I Xba I EcoR I Stu I

1090

ACTAGTCGGCCGTACGGGCCC

Spe I BsiW I Bsp120 I Apa I

Restriction Map and Multiple Cloning Site (MCS) of pEGFP Vector. Unique restriction sites are in bold. The *Xba* I sites in the MCS can be used together to excise the EGFP gene.

Description:

pEGFP carries a red-shifted variant of wild-type green fluorescent protein (GFP) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP encodes the GFPmut1 variant (1) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (2). Upstream sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (3) to further increase the translation efficiency in eukaryotic cells.

The EGFP gene was cloned between the two MCS of the pUC19 derivative pPD16.43 (4). The EGFP coding sequence is flanked by separate MCS at the 5' and 3' ends, so the EGFP gene can be easily excised from pEGFP. Alternatively, the EGFP coding sequence can be amplified by PCR. The EGFP gene was inserted in frame with the *lacZ* initiation codon from pUC19 so that a EGFP fusion protein is expressed from the *lac* promoter in *E. coli.* Note, however, that if you excise the EGFP coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., non-fusion) EGFP protein. The pUC backbone of EGFP provides a high-copy-number origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli.*

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(PR29965; published 03 October 2002)

Location of features:

- *lac* promoter: 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - Transcription start point: 179
  - *lac* operator: 179–199
- *lacZ*–EGFP fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; Stop codon: 1006–1008
- 5' Multiple Cloning Site: 234–281
- Enhanced green fluorescent protein (EGFP) gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; Stop codon: 1006–1008
  - Insertion of Val at position 2: 292–294
  - GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 481–486
  - His-231 to Leu mutation (A→T): 983
- 3' Multiple Cloning Site: 1010–1109
- Ampicillin resistance gene
  - Promoter: –35 region: 1485–1490; –10 region: 1508–1513
  - Transcription start point: 1520
  - Ribosome binding site: 1543–1547
  - β-lactamase coding sequences:
    - Start codon (ATG): 1555–1557; Stop codon: 2413–2415
    - β-lactamase signal peptide: 1555–1623
    - β-lactamase mature protein: 1624–2412
- pUC plasmid replication origin: 2563–3206

Primer location:

- EGFP-N Sequencing Primer (#6479-1): 355–334
- EGFP-C Sequencing Primer (#6478-1): 942–963

Propagation in *E. coli*:

- Recommended host strain: JM109
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. Fire, A., *et al.* (1990) *Gene* **93**:189–198.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

GE Healthcare

Material Safety Data Sheet

Canada
English

Section 1. Chemical product and company identification

Product name pGEX-4T-1, 25 µg

Catalogue Number 27-4580-01

Material uses	Industrial applications: Analytical chemistry. Research.
Product type	Liquid.
Validation date	16 April 2009
Print date	16 April 2009
Supplier	GE Healthcare UK Ltd Amersham Place Little Chalfont Buckinghamshire HP7 9NA England +44 0870 606 1921

In case of emergency			
	US	ChemTrec (US)	1-800-424-9300
	Canada	ChemTrec (US)	1-703-527-3887

2. Hazards identification

Physical state	Liquid.
Odor	Odorless.
Emergency overview	No specific hazard. NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED. No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
Routes of entry	Dermal contact. Eye contact. Inhalation. Ingestion.

Potential acute health effects

Eyes	No known significant effects or critical hazards.
Skin	No known significant effects or critical hazards.
Inhalation	No known significant effects or critical hazards.
Ingestion	No known significant effects or critical hazards.

Potential chronic health effects

Chronic effects	No known significant effects or critical hazards.
Carcinogenicity	No known significant effects or critical hazards.
Mutagenicity	No known significant effects or critical hazards.
Teratogenicity	No known significant effects or critical hazards.
Developmental effects	No known significant effects or critical hazards.
Fertility effects	No known significant effects or critical hazards.
Target organs	Not available.
Inhalation	No specific data.
Ingestion	No specific data.
Skin	No specific data.
Eyes	No specific data.
Medical conditions aggravated by over-exposure	None known.

See toxicological information (section 11)

3. Composition/information on ingredients

There are no ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

Section 4. First aid measures

Eye contact	Check for and remove any contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical attention if symptoms occur.
Skin contact	In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if symptoms occur.
Inhalation	Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms occur.
Ingestion	Wash out mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if symptoms occur.
Protection of first-aiders	No action shall be taken involving any personal risk or without suitable training.

Section 5. Fire fighting measures

Flammability of the product	In a fire or if heated, a pressure increase will occur and the container may burst.
Extinguishing media	
Suitable	Use an extinguishing agent suitable for the surrounding fire.
Not suitable	None known.
Special exposure hazards	Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable training.
Special protective equipment for fire-fighters	Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Section 6. Accidental release measures

Personal precautions	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Put on appropriate personal protective equipment (see section 8).
Environmental precautions	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
Methods for cleaning up	Stop leak if without risk. Move containers from spill area. Prevent entry into sewers, water courses, basements or confined areas. Wash spillages into an effluent treatment plant or proceed as follows. Contain and collect spillage with non-combustible, absorbent material e.g. sand, earth, vermiculite or diatomaceous earth and place in container for disposal according to local regulations (see section 13). Dispose of via a licensed waste disposal contractor. Note: see section 1 for emergency contact information and section 13 for waste disposal.
Small spill	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.

Section 7. Handling and storage

Handling	Put on appropriate personal protective equipment (see section 8). Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking.
Storage	Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully reseaIed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

Section 8. Exposure controls/personal protection

Consult local authorities for acceptable exposure limits.

Recommended monitoring procedures	If this product contains ingredients with exposure limits, personal, workplace atmosphere or biological monitoring may be required to determine the effectiveness of the ventilation or other control measures and/or the necessity to use respiratory protective equipment.
Engineering measures	No special ventilation requirements. Good general ventilation should be sufficient to control worker exposure to airborne contaminants. If this product contains ingredients with exposure limits, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure below any recommended or statutory limits.

Hygiene measures	Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

Personal protection

Respiratory	Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.
Hands	Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.
Eyes	Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists or dusts.
Skin	Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.
Environmental exposure controls	Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

Section 9. Physical and chemical properties

Physical state	Liquid.
Color	Colorless.
Odor	Odorless.
pH	7.5 [Conc. (% w/w): 100%]
Volatility	0% (v/v)
VOC	0 (g/l).
Solubility	Easily soluble in the following materials: cold water and hot water.

Section 10. Stability and reactivity

Stability	The product is stable.
Materials to avoid	No specific data.
Hazardous polymerization	Under normal conditions of storage and use, hazardous polymerization will not occur.
Conditions of reactivity	Non-flammable in the presence of the following materials or conditions: open flames, sparks and static discharge, heat, shocks and mechanical impacts, oxidizing materials, reducing materials, combustible materials, organic materials, metals, acids, alkalis and moisture. Not considered to be a product presenting a risk of explosion.

Section 11. Toxicological information

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Not available.				

Conclusion/Summary Not available.

Classification

Product/ingredient name	ACGIH	IARC	EPA	NIOSH	NTP	OSHA
Not available.						

Synergistic products Not available.

Section 12. Ecological information

Environmental effects	No known significant effects or critical hazards.
Octanol/water partition coefficient	Not available.
Bioconcentration factor	Not available.
Other adverse effects	No known significant effects or critical hazards.

Section 13. Disposal considerations

Waste disposal	The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in a safe way. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.
RCRA classification	Not available.

Disposal should be in accordance with applicable regional, national and local laws and regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

Section 14. Transport information

International transport regulations

Not classified.

Section 15. Regulatory information

WHMIS (Canada)	Not controlled under WHMIS (Canada).
Canadian lists	**CEPA Toxic substances:** None of the components are listed. **Canadian ARET:** None of the components are listed. **Canadian NPRI:** None of the components are listed. **Alberta Designated Substances:** None of the components are listed. **Ontario Designated Substances:** None of the components are listed. **Quebec Designated Substances:** None of the components are listed.
Canada inventory	All components are listed or exempted.

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

EU regulations

Hazard symbol or symbols

Risk phrases	This product is not classified according to EU legislation.
Safety phrases	Not applicable.

International regulations

International lists	**Australia inventory (AICS):** All components are listed or exempted. **China inventory (IECSC):** All components are listed or exempted. **Japan inventory (ENCS):** All components are listed or exempted. **Japan inventory (ISHL):** Not determined. **Korea inventory (KECI):** All components are listed or exempted. **New Zealand Inventory of Chemicals (NZIoC):** All components are listed or exempted. **Philippines inventory (PICCS):** All components are listed or exempted.

Section 16. Other information

The customer is responsible for determining the PPE code for this material.

Indicates information that has changed from previously issued version.

History

Date of printing	16 April 2009	**Date of previous issue**	25 July 2006
Date of issue	16 April 2009	**Version**	3

Notice to reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

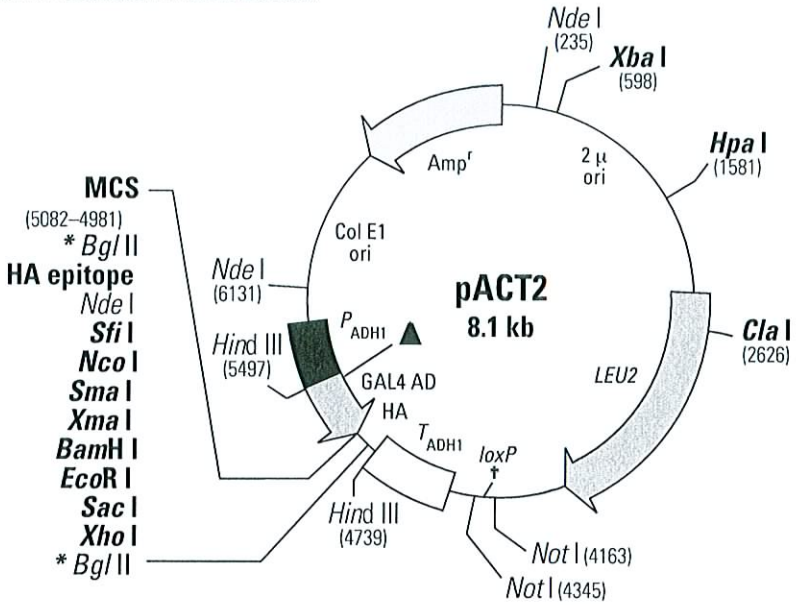
Vector Information

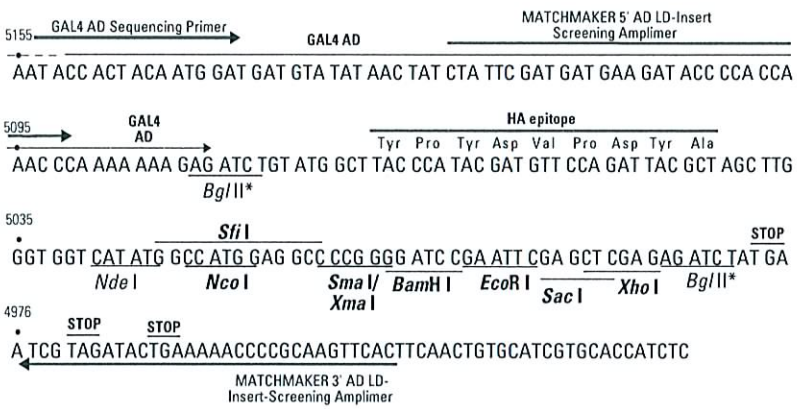
pACT2 AD Vector Information

PT3022-5

GenBank Accession No.: U29899.

Catalog 638822





Restriction Map and Multiple Cloning Site (MCS) of pACT2 AD. Unique restriction sites are in bold.

Description:

pACT2 generates a fusion of the GAL4 AD (amino acids 768–881), an HA epitope tag, and a protein of interest (or protein encoded by a cDNA in a fusion library) cloned into the MCS in the correct orientation and reading frame. pACT2, which is derived from pACT (1), contains a unique *Eco*R I site in the MCS. The hybrid protein is expressed at high levels in yeast host cells from the constitutive ADH1 promoter (*P*); transcription is terminated at the ADH1 transcription termination signal (*T*). The protein is targeted to the yeast nucleus by the nuclear localization sequence from SV40 T-antigen which has been cloned into the 5' end of the GAL4 AD sequence (2). pACT2 is a shuttle vector that replicates autonomously in both *E. coli* and *S. cerevisiae* and carries the *bla* gene, which confers ampicillin resistance in *E. coli*. pACT2 also contains the *LEU2* nutritional gene that allows yeast auxotrophs to grow on limiting synthetic media. Transformants with AD/library plasmids can be selected by complementation by the *LEU2* gene by using an *E. coli* strain that carries a *leuB* mutation (e.g., HB101).

Note: The *Sfi* I and *Sma* I sites in the MCS tend to compress during sequencing.

(PR8X956)

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Location of features:

- 2μ origin of replication: 1–2055
- LEU2 coding sequences
  Start codon (ATG): 2474–2476
  Stop codon: 3566–3568
- Lox sites: Lox 1: 4268–4327; Lox 2: 4367–4412
- Transcription termination signal
  Fragment carrying the *S. cerevisiae* ADH1 terminator: 4415–4742
  Translation stop codon: 4970–4972
- Multiple cloning site: 4927–5079
- Hemagglutinin (HA) epitope: 5042–5068
- GAL4 activation domain polypeptide
  Start codon (ATG): 5486–5488
  GAL4 codons 768–881: 5081–5419
  SV40 T-antigen nuclear localization signal: 5424–5478
- Promoter fragment carrying the *S. cerevisiae* ADH1 promoter: 5504–5901
- pBR322 plasmid replication origin: 6336–6979
- Ampicillin resistance gene
  Promoter: –35 region: 8052–8057; –10 region: 8029–8034
  Transcription start point: 8022
  Ribosome binding site: 7995–7999
  β-lactamase coding sequences:
  Start codon (ATG): 7985–7987
  Stop codon: 7127–7129
  β-lactamase signal peptide: 7919–7987
  β-lactamase mature protein: 7130–7918

Primer locations:

- MATCHMAKER 5' LD-Insert Screening Amplimer: 5122–5091
- MATCHMAKER 3' LD-Insert Screening Amplimer: 4943–4974
- GAL4 AD Sequencing Primer: 5153–5137

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pBR322
- Copy number: 15–20

Propagation in *S. cerevisiae*

- Suitable host strains: Y187(α), Y190 (a), SFY526(a), CG1945(a), or HF7c(a)
- Selectable marker: LEU2
- *S. cerevisiae* replication origin: 2μ
- Copy number: multiple copy

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

MATERIAL SAFETY DATA SHEET

Revised 2/10/04
Replaces 11/17/03
Printed 2/10/04

PLASMID PFASTBAC DUAL
INVITROGEN CORPORATION
MSDS ID: 10712

1. PRODUCT AND COMPANY INFORMATION

INVITROGEN CORPORATION
1600 FARADAY AVE.
CARLSBAD, CA 92008
760/603-7200

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716/774-6700

INVITROGEN CORPORATION
3 FOUNTAIN DR.
INCHINNAN BUSINESS PARK
PAISLEY, PA4 9RF
SCOTLAND
44-141 814-6100

INVITROGEN CORPORATION
P.O. BOX 12-502
PENROSE
AUCKLAND 1135
NEW ZEALAND
64-9-579-3024

INVITROGEN CORPORATION
2270 INDUSTRIAL ST.
BURLINGTON, ONT
CANADA L7P 1A1
905/335-2255

INVITROGEN AUSTRALIA PTY LIMITED
2A/14 LIONEL ROAD
MOUNT WAVERLY VIC 3149
AUSTRALIA
1-800-331-627

EMERGENCY NUMBER (SPILLS, EXPOSURES): 301/431-8585 (24 HOUR)
800/451-8346 (24 HOUR)
NON-EMERGENCY INFORMATION: 800/955-6288

Product Name: PLASMID PFASTBAC DUAL
Stock Number: 10712

NOTE: If this product is a kit or is supplied with more than one material, please refer to the MSDS for each component for hazard information.

Product Use:
These products are for laboratory research use only and are not intended for human or animal diagnostics, therapeutic, or other clinical uses.

Synonyms:
Not available.

2. COMPOSITION, INFORMATION ON INGREDIENTS

The following list shows components of this product classified as hazardous based on physical properties and health effects:

Component	CAS No.	Percent
No hazardous components		

3. HAZARDS IDENTIFICATION

********** EMERGENCY OVERVIEW **********
Occupational exposure presents little or no health hazard.
****

Potential Health Effects:
Eye:
May cause irritation of the eye.

Skin:
May cause skin irritation.

Inhalation:
No toxicity expected from inhalation.

Ingestion:
Mildly irritating to mouth, throat, and stomach. Can cause abdominal discomfort.

Chronic:
No data on cancer.

4. FIRST AID MEASURES

Eye:
Flush with water in an eyewash for at least 15 minutes, holding eyelids open. Remove contact lenses, clean before re-use. Obtain medical attention if symptoms develop (redness, itching, etc.).

Skin:
Wash with plenty of soap and water. Obtain medical attention if symptoms develop (redness, itching, etc.). Remove contaminated clothes, wash before re-use.

Inhalation:
Remove person to fresh air. Obtain medical attention unless effects are mild and temporary.

Ingestion:
Give plenty of water, if conscious. If vomiting occurs naturally, wash mouth out. Be prepared to induce vomiting upon a physician's advice. Obtain medical attention if symptoms develop.

Note To Physician:
Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flashpoint Deg C:
Not available.

Upper Flammable Limit %:
Not available.

Lower Flammable Limit %:
Not available.

Autoignition Temperature Deg C:
Not available.

Extinguishing Media:
Use means appropriate for surrounding materials.

Firefighting Techniques/Equipment:
Standard turnout gear ("bunker gear"). Positive-pressure self-contained breathing apparatus.

Hazardous Combustion Products:
Includes carbon dioxide, carbon monoxide, dense smoke.

6. ACCIDENTAL RELEASE MEASURES

Accidental releases may be subject to special reporting requirements and other regulatory mandates. Refer to Section 8 for personal protection equipment recommendations.

Spill Cleanup:
Absorb spill. Common absorbent materials should be effective. Deposit in appropriate containers for removal and disposal.

7. HANDLING AND STORAGE

Storage of some materials is regulated by federal, state, and/or local laws.

Storage Pressure:
Ambient

Handling Procedures:
Keep closed or covered when not in use.

Storage Procedures:
Suitable for most general chemical storage areas.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

Exposure Limits:

Component	OSHA PEL (ppm)	AGCIH TWA (ppm)
No hazardous components		

Engineering Controls:
Area ventilation is generally adequate.

Personal Protective Equipment:

Eye:
Safety glasses should be the minimum eye protection.

Skin:
Gloves should be used as minimum hand protection.

Respiratory:
No respiratory protection will be needed under normal industrial operating conditions.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance/physical state:	Clear, colorless liquid.
Odor:	No odor.
Boiling Point (C):	100
Melting Point (C):	0
Solubility in water:	100%
pH:	7.0
Vapor Pressure:	0.0212 ATM AT 20°C
Vapor Density:	17.3 G/M3
Specific Gravity/Density:	1.00
Octanol/water Partition Coeff:	Not established.
Volatiles:	Not established.
Evaporation Rate:	0.3 (BUTYL ACETATE = 1.0)
Viscosity:	0.98 cp at 20°C

10. STABILITY AND REACTIVITY

Stability:
Stable under ordinary conditions of use and storage.

Conditions to Avoid:
Strong oxidizers.

Hazardous Decomposition Products:

10. STABILITY AND REACTIVITY (CONT.)

None known.

Hazardous Polymerization:
Not expected to occur.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity:

Dermal/Skin:
Not determined.

Inhalation/Respiratory:
Not determined.

Oral/Ingestion:
Not determined.

Target Organs: No data found.

Carcinogenicity:

NTP:
Not tested.

IARC:
Not listed.

OSHA:
Not regulated.

Other Toxicological Information

12. Ecological Information

Ecotoxicological Information: No ecological information available.

Environmental Fate (Degradation, Transformation, and Persistence):
Bioconcentration is not expected to occur.

13. DISPOSAL CONSIDERATIONS

Regulatory Information:
Not applicable.

13. DISPOSAL CONSIDERATIONS (CONT.)

Disposal Method:
Clean up and dispose of waste in accordance with all federal, state, and local environmental regulations.

14. TRANSPORT INFORMATION

Proper Shipping Name: Not Determined.
Subsidiary Hazards:

15. REGULATORY INFORMATION

UNITED STATES:

TSCA:
This product is solely for research and development purposes only and may not be used, processed or distributed for a commercial purpose. It may only be handled by technically qualified individuals.

Prop 65 Listed Chemicals: PROP 65 PERCENT
No Prop 65 Chemicals.

No 313 Chemicals

CANADA:

DSL/NDSL:
Not determined.

COMPONENT WHMIS Classification
No hazardous components

EUROPEAN UNION:

PRODUCT RISK PHRASES:
None assigned.

PRODUCT SAFETY PHRASES:
Not applicable.

PRODUCT CLASSIFICATION:
Not classified as hazardous

Component EINECS Number
No hazardous components

16. OTHER INFORMATION

HMIS Rating 0-4:
FIRE: Not determined.
HEALTH: Not determined.
REACTIVITY: Not determined.

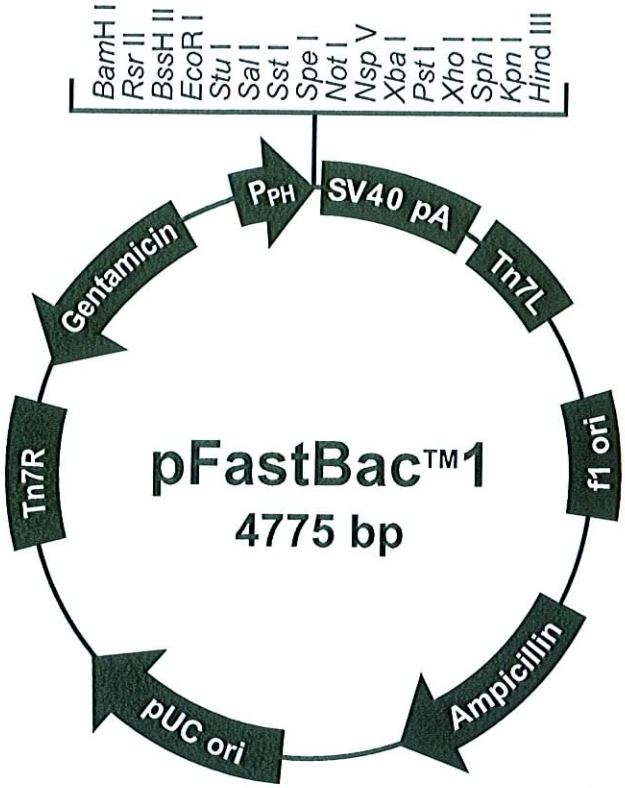
Abbreviations
N/A - Data is not applicable or not available
SARA - Superfund and Reauthorization Act
HMIS - Hazard Material Information System
WHMIS - Workplace Hazard Materials Information System
NTP - National Toxicology Program
OSHA - Occupational Health and Safety Administration
IARC - International Agency for Research on Cancer
PROP 65 - California Safe Drinking Water and Toxic Enforcement Act of 1986
EINECS - European Inventory of Existing Commercial Chemical Substances

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

Map and Features of pFastBac™1

pFastBac™1 Map

The map below shows the elements of pFastBac™1. **The vector sequence of pFastBac™1 is available from our website (www.invitrogen.com) or by contacting Technical Support (see page 66).**



Comments for pFastBac™1
4775 nucleotides

f1 origin: bases 2-457
Ampicillin resistance gene: bases 589-1449
pUC origin: bases 1594-2267
Tn7R: bases 2511-2735
Gentamicin resistance gene: bases 2802-3335 (complementary strand)
Polyhedrin promoter (P_{PH}): bases 3904-4032
Multiple cloning site: bases 4037-4142
SV40 polyadenylation signal: bases 4160-4400
Tn7L: bases 4429-4594

Continued on next page

Map and Features of pFastBac™1, continued

Features of the Vector

pFastBac™1 (4775 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
Polyhedrin promoter (P_{PH})	Allows efficient, high-level expression of your recombinant protein in insect cells (O'Reilly *et al.*, 1992).
Multiple cloning site	Allows restriction enzyme-mediated cloning of your gene of interest.
SV40 polyadenylation signal	Permits efficient transcription termination and polyadenylation of mRNA (Westwood *et al.*, 1993).
Tn7L and Tn7R	Mini Tn7 elements that permit site-specific transposition of the gene of interest into the baculovirus genome (*i.e.*, bmon14272 bacmid) (Luckow *et al.*, 1993).
f1 origin	Allows rescue of single-stranded DNA.
Ampicillin resistance gene	Allows selection of the plasmid in *E. coli*.
pUC origin	Permits high-copy replication and maintenance in *E. coli*.
Gentamicin resistance gene	Permits selection of the recombinant bacmid in DH10Bac™ *E. coli*.

How we make recombinant adenovirus.

VIROLOGY 163, 614–617 (1988)

SHORT COMMUNICATIONS

A Simple Technique for the Rescue of Early Region I Mutations into Infectious Human Adenovirus Type 5

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Early region 1 (E1) of the human adenoviruses has many intriguing properties which have prompted numerous mutational studies to help delineate and characterize the domains responsible for these functions. In mutational analyses being done currently, the E1 region is usually cloned into a bacterial plasmid where it is mutated and then the altered E1 sequences are "rescued" back into infectious virus. The most frequently used rescue procedures are somewhat tedious, requiring the purification and fractionation of linear viral DNA or DNA fragments, and often involve the screening of numerous plaque isolates. Several observations we have made recently on the properties of adenovirus DNA in infected cells and on infectious plasmids in transfected cells led us to design a new approach for rescuing E1 mutations into infectious viral genomes. We constructed a plasmid, pJM17, containing the entire Ad5 DNA molecule, with an insert in the E1 region that exceeds the packaging constraints of the adenovirus capsid. Following transfection of pJM17 into 293 cells the plasmid DNA is able to replicate but cannot be packaged into infectious virions. In contrast cotransfection of 293 cells with pJM17 plus an E1-containing plasmid carrying mutated sequences produces recombinant virions at high efficiencies. Neither plasmid needs to be linearized prior to cotransfection. The technique eliminates the need to purify and manipulate infectious virion DNA and since no unique restriction sites are needed, both E1A and E1B mutants as well as foreign gene inserts in the E1 region can be easily rescued into virus.

Early region 1 (E1) of the human adenoviruses has a number of interesting activities. First and foremost, this region is necessary, and apparently sufficient, for oncogenic transformation of cells in culture and plays a pivotal and complex role in regulation of the viral lytic cycle (see Refs *1* and *2* for review). E1A functions appear to be able to "immortalize" primary rodent cells following DNA-mediated gene transfer and can "cooperate" with other oncogenes, such as E1B or Ha-*ras* to induce oncogenic transformation (3, 4). One of the key actions of E1A proteins seems to be at the level of regulation of gene expression. E1A gene products have been shown to activate transcription of other early viral regions (E1B, E2, E3, E4, and L1) as well as certain cellular genes and can stimulate transcription of cellular genes which have been cotransfected into mammalian cells (*2, 5, 6*). Finally, E1A products seem to be capable of repressing the enhancement of transcription of some cellular genes (*2, 5–7*).

These intriguing properties have generated intense interest in the proteins encoded by E1A and many groups are involved in the generation and characterization of mutants in this region. One of the approaches being followed is the construction of mutations in E1A sequences cloned in bacterial plasmids using a variety of recombinant DNA techniques. Such mutants can be studied by use of DNA-mediated gene transfer to introduce them into mammalian cells but ultimately it is desirable to "rescue" the mutations made in cloned DNA back into infectious virus. To this end a number of techniques are available, the most widely used being those based on the method of Stow (*8*) in which viral DNA is cut with a restriction enzyme once near the left of the genome and the large fragment is either ligated to or cotransfected with plasmid DNA containing mutated E1A sequences. Besides the need to work with linear viral DNA with an inherently low specific activity, this approach has the disadvantage that it can sometimes involve the screening of large numbers of plaque isolates in order to identify the desired viral mutant. This can become tedious if large numbers of mutants are being rescued.

We have recently made several observations which suggested a better way to obtain recombinant viruses. First, we have shown that adenovirus DNA circularizes in infected cells (*9*) and have used this phenomenon to clone adenovirus genomes as bacterial plasmids which generate infectious virus following transfection of mammalian cells (*10, 11*). Second, we have found that cotransfected plasmids can recombine efficiently in mammalian cells without the need to linearize either plasmid and that this process can generate infectious virus from two noninfectious plasmids (*12*). And third,

[1] To whom requests for reprints should be addressed.

0042-6822/88 $3.00

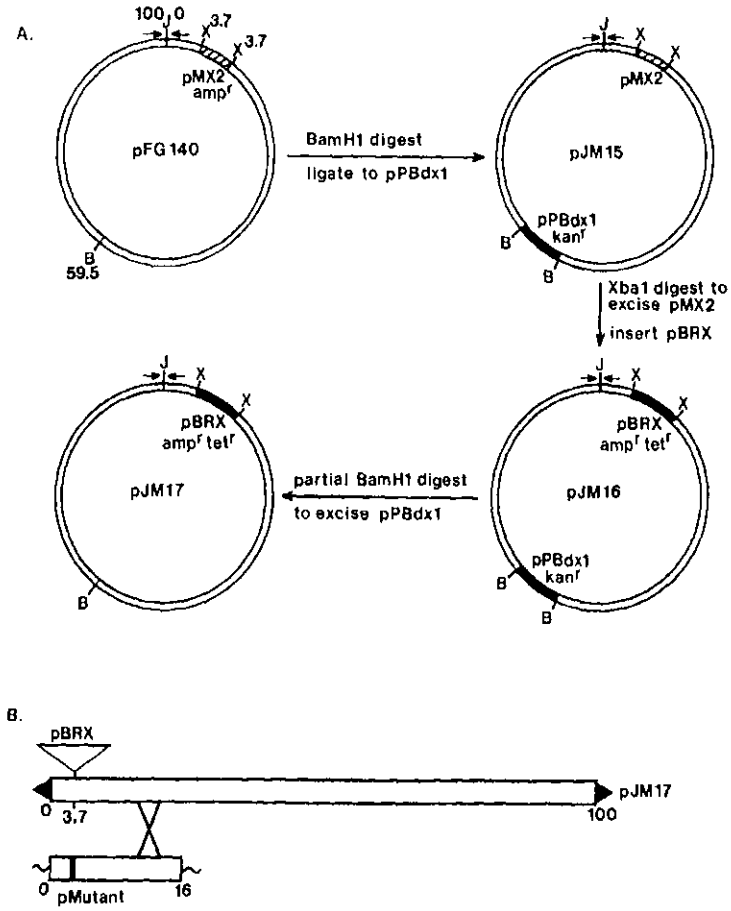

FIG. 1. The construction of pJM17 and its use in the rescue of E1 mutants. (A) The infectious Ad5 plasmid pFG140, containing the 2.2-kb ampicillin resistant plasmid pMX2 inserted at the unique *Xba*I site at 3.7 m.u. of the Ad5 genome, was restricted with *Bam*HI and ligated to the kanamycin resistant plasmid pPBdx1 (*11*) to give pJM15. The pMX2 insert was excised by *Xba*I digestion followed by insertion of a 4.3-kb plasmid, pBRX (*24*) encoding ampicillin and tetracycline resistance, at the unique Ad5 *Xba*I site. The resulting plasmid, pJM16, was then partially digested with *Bam*HI to remove pPBdx1 yielding pJM17 of approximate size 40.3 kb. J denotes the junction of the left and right ends of the viral genome, X and B denote *Xba*I and *Bam*HI restriction sites. Relevant positions on the Ad5 genome are indicated in map units (100 m.u. = 36 kb). (B) A possible mechanism for the *in vivo* recombination event between pJM17 and a plasmid containing a mutation in E1. It should be noted that although both plasmids in this drawing are represented as linear they are in fact transfected as circular DNA. In pJM17 the ends of viral DNA are joined (indicated by arrows) whereas the E1 sequences in the pMutant DNA are joined by sequences derived from pUC19. pJM17 DNA may become linearized in transfected 293 cells prior to recombination with overlapping sequences from the plasmid containing E1.

we have shown that the maximum amount of viral DNA which can be packaged into capsids is only approximately 2 kb in excess of the wild-type genome size (*12*).

These observations led us to design and execute the strategy shown in Fig. 1. The infectious plasmid pFG140 (*10*) was modified so that instead of containing a 2.2-kb insert (pMX2) at 3.7 m.u. we substituted a 4.3-kb insert (pBRX) to create the plasmid pJM17. Because pJM17 is about 2 kb larger than the maximum amount of DNA which can be packaged into adenovirus capsids (*12*) it can give rise only to infectious virus following rearrangements which excise all or part of the pBRX DNA. This process occurs inefficiently and gives rise to small plaques at low frequency, and only after long incubation times. In contrast, cotransfection of pJM17 with a plasmid containing the left end of Ad5 can give rise to infectious virus very efficiently. Plaques generated by this process are normal in size and time of appearance and contain virus having the structure predicted to arise by homologous *in vivo* recombination between the two plasmids as indicated in Fig. 1B. We do not know either at what stage or by what mechanism recombination occurs. A likely possibility is that following transfection of 293 cells pJM17 DNA proceeds to replicate as linear viral DNA and that

recombinants are generated by the same process as when linear viral DNA and left-end sequences are cotransfected into cells.

As part of a program of study of E1A functions we have constructed a large number of linker insertion mutations in E1A sequences cloned in a bacterial plasmid (Bautista, McGrory, and Graham, unpublished observations) and are using cotransfection with pJM17 routinely to rescue the mutations into infectious virus. Because the inserted linkers are flanked by *Bam*HI restriction sites, screening for recombinant virus is done simply by digestion of the viral DNA with the enzymes *Hin*dIII and *Bam*HI which generate easily interpretable restriction patterns on agarose gels. Using such E1A mutants we have tested and optimized the mutant rescue procedure outlined schematically in Fig. 1B. First the efficiency of plaque formation as a function of the cotransfected plasmid DNA concentrations was measured to determine the optimum concentrations of DNA for cotransfection. The results of two experiments (Table 1) show that the best plaque yield is from an equimolar amount of the two plasmids or with a slight excess of the smaller plasmid. In separate experiments (data not shown) we have found that pJM17 alone is slightly infectious at high concentrations due to rearrangements of the viral genome reducing it to a packageable size. However, the plaquing efficiency is very low with pJM17 alone; the plaques are relatively small, and take about twice as long for a productive infection on 293 cells as compared to plaques from cotransfections. The low level of background infectivity of pJM17 is overshadowed by cotransfection with the smaller E1 plasmid presumably because recombination between cotransfected plasmid DNA is more efficient than rearrangement of pJM17 sequences and because recombinants replicate faster than progeny derived from rearrangement. Progeny derived from pJM17 alone can be further reduced by selecting only the largest plaques for screening following cotransfections of 293 cells.

Screening of 68 plaques from three separate experiments showed a rescue success of 70 to 80% regardless of the relative amounts of the two cotransfecting plasmids. The efficiency of rescue was not affected by the location of the mutation relative to the pBRX insert in the sense that E1 mutations to the left or right of 3.7 m.u. could be readily rescued. In our experiments the mutants were contained in an E1 plasmid extending to the *Xho*I site at 16 m.u. and we have shown the technique can rescue an E1B mutation with an insertion at nucleotide 1969 with high efficiency. The rescue technique also worked for the rescue of E1A mutations contained on a plasmid having Ad5 DNA sequences extending only to the *Kpn*I site at 2050 (5.8 m.u.). However, in these experiments the efficiency was lower (approximately 30 to 40%) probably as a result of the decreased overlap in homologous DNA sequences available for recombination rightward of the pBRX insertion.

Results obtained to date illustrate the convenience of this approach for the rescue of E1 mutants into infectious virus. The ability to work with the Ad5 viral genome entirely in bacteria prior to the actual rescue into virus is an economical and time-saving advantage. Our technique also has applications in other areas of research such as the use of adenovirus as a eucaryotic vector in the study of foreign genes. Numerous foreign genes, such as those coding for the polyoma

TABLE 1

PLAQUING EFFICIENCY OF pJM17 AND E1 MUTANT PLASMIDS COTRANSFECTED ON 293 CELLS

pJM17 (μg/dish)[a]	E1 plasmid: pX1056 (μg/dish)					E1 plasmid: pX1376 (μg/dish)				
	0	0.5	1.0	2.0	5.0	0	0.1	0.25	1.0	2.0
0.5	0[b]	1	1	0	1	0	1	0	2	1
1.0	0	2	6	11	5	0	3	1	5	5
2.0	0	5	9	10	11	0	0	5	4	4
5.0	8[c]	13	32	35	37	0	3	4	13	18

[a] pJM17 and the indicated mutant E1 plasmid were cotransfected onto 293 cells at various plasmid DNA concentrations using the calcium technique (*25*). Plasmid pX1056 contains the left 16 m.u. of Ad5 DNA and a *Bam*HI linker insertion mutation at nucleotide 1056 (2.9 m.u.) and pX1376 contains a similar mutation at nucleotide 1376 (3.8 m.u.).

[b] Total number of plaques scored at 11 days post-transfection from two 60-mm dishes.

[c] Small plaques which resulted from deletion of sequences in and around the pBRX insert in pJM17.

virus middle tumor antigen (*13–16*), mouse dihydrofolate reductaste (*17*), the hepatitis B surface antigen (*18, 19, 20*), the SV40 tumor antigen (*21, 22*), and herpes simplex virus 1 (HSV-1) thymidine kinase (*23*) have been inserted at various sites in the Ad5 genome and in some cases have been shown to express levels of protein at comparable or increased rates relative to the wild-type expression of the genes. The rescue technique described here has recently been used to clone the HSV-2 ribonucleotide reductase gene into Ad5 (S. Bacchetti, personal communication) and an analogous orocedure has recently been employed successfully to insert the HSV-1 glycoprotein B gene into the E3 region of Ad5 (D. Johnson, personal communication)

ACKNOWLEDGMENTS

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Describes genetic defects in the viral dl309 background

ELSEVIER

Virus Research 39 (1995) 75–82

Virus Research

DNA sequence of the deletion/insertion in early region 3 of Ad5 *dl*309 ☆

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Abstract

*dl*309 is an adenovirus type 5 (Ad5) mutant that has been extensively utilized for construction of Ad5 mutants in early region 1 (E1), in developing vectors for use as viral vaccines, and in development of gene transfer vectors for gene therapy. Ad5 *dl*309 has been useful for vector construction because of its altered *Xba*I restriction pattern and lends itself to a variety of strategies for rescuing inserts or mutations into E1. It contains only one *Xba*I site at 3.7 map units (m.u.) as compared to wt Ad5 which contains 4 (3.7, 29.5, 79.5, and 84.8 m.u.). The loss of the sites at 29.5 and 79.5 m.u. is due to deletions of a few bp but the loss of the site at 84.8 m.u. was the result of a deletion from approximately 83 to 85 m.u. and substitution with a fragment of foreign DNA. Because of the widespread use of *dl*309 and derivatives of this mutant in the construction of Ad5-based vectors and the need to have precise genetic information on the sequences present in vectors to be used as vaccines and in gene therapy, we have sequenced the alterations in *dl*309 which affect the *Xba*I sites at 79.5 and 84.8 m.u. and have determined which E3 proteins are expressed by this virus. The deletion that removes the *Xba*I site at 84.8 m.u. extends from Ad5 bp 30005–30750 and is substituted with 642-bp of heterologous DNA that shows homology to salmon DNA. This alteration deletes all or part of the coding sequences for the E3 14.7K, 14.5K and 10.4K proteins and these proteins were not detected in *dl*309 infected cells. The loss of the *Xba*I site at 79.5 m.u. is the result of a 6-bp deletion which removes two internal amino acids (18 and 19) from the E3 6.7K protein. The E3 6.7K protein and other E3 proteins whose coding

☆ The accession number for the insertion/deletion in *dl*309 is U22898.

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sequences are unaffected by the alterations in *dl*309 (gp19K, 12.5K and 11.6K) were expressed in *dl*309 infected cells.

Keywords: Adenovirus; Viral vector; Early region 3; DNA sequence; Prolactin

Adenoviruses (Ads) have been used extensively to express heterologous proteins in mammalian cells (Graham and Prevec, 1991, 1992; Berkner, 1992) and are currently attracting considerable attention because of their potential use as live viral vectored vaccines (Berkner, 1992; Graham and Prevec, 1992) and as gene transfer vectors in gene therapy (Siegfreid, 1993; Trapnell, 1993). A number of strategies have been used for the construction of Ad vectors for these purposes, all of which involve the manipulation of subgenomic fragments of the Ad genome (Graham and Prevec, 1991; Berkner, 1992). Due to the relatively large size of the Ad5 genome and the lack of unique restriction sites that can be used in vector construction, *dl*309 (Jones and Shenk, 1979), an Ad5 mutant with an altered *Xba*I restriction pattern, has been extensively used in place of wt Ad5. This mutant contains only one (at 3.7 map units (m.u.)) of the 4 (3.7, 29.5, 79.5, and 84.8 m.u.) *Xba*I sites present in the wt genome.

*dl*309 was obtained by selection for variants in a wt Ad5 population that lacked restriction endonuclease sites (Jones and Shenk, 1978, 1979). Briefly Ad5 DNA was digested with the restriction enzyme of interest and the DNA was religated then transfected into 293 cells (Graham et al., 1977) to obtain viral plaques which were then screened for mutants lacking cleavage sites. This procedure enriched for variants in the viral population that had a reduced number of cleavage sites, since fewer ligation events were required to regenerate the genome (Jones and Shenk, 1978, 1979). Using this procedure *dl*309 was obtained in two steps. First variants were selected which lacked one of the *Eco*RI restriction sites, present at 76.1 and 83.6 m.u. in wt Ad5 DNA. This resulted in the isolation of *sub*304 (Jones and Shenk, 1978) which had lost the *Eco*RI site at 83.6 m.u. due to a deletion from approximately 83–85 m.u. and substitution with foreign DNA of unknown origin. Because the deletion in *sub*304 also removed the *Xba*I site at 84.8 m.u. this mutant was then used to select for viruses that lacked additional *Xba*I restriction sites (Jones and Shenk, 1979). Successive rounds of selection generated mutant 308 (missing the *Xba*I site at 79.6 m.u.) which was then used in a further round of selection to obtain mutant 309 which had lost the *Xba*I site at 29.5 m.u. Because of the position of the remaining *Xba*I site at 3.7 m.u., *dl*309 DNA has been used in direct ligation (Stow, 1981) and cotransfection strategies (Berkner and Sharp, 1983; Haj-Ahmad and Graham, 1986) for rescuing mutations or inserts into early region 1 and was the parent virus for the development of a number of systems for Ad5 vector construction based on bacterial plasmids (Graham, 1984; Ghosh-Choudhury et al., 1986; McGrory et al., 1988).

To sequence the alteration at 79.6 m.u. and the deletion/insertion between 83 and 85 m.u., pFG140 (Graham, 1984) (derived from *dl*309) was partially digested with *Xho*I and religated to generate pFGdX8. pFGdX8 contains *dl*309 sequences

from bp 4 (left genomic end) to bp 5788 (m.u. 16.1) and from bp 24796 (m.u. 69.0) to the right genomic end save for the last 10-bp. The left and right termini of the genome are covalently joined and an ampicillin resistant plasmid pMX2 inserted at the *Xba*I site at bp 1339 allows propagation of pFGdX8 in *Escherichia coli*. DNA sequencing reactions were based on the chain-termination method (Sanger et al., 1977) and manual sequencing following the DNA sequencing protocol described in the Sequenase™ kit produced by US Biochemical. [α-^{32}P]dATP was obtained from Amersham Canada Ltd. All oligonucleotide primers were synthesized by the central facility of the Molecular Biology and Biotechnology Institute (MOBIX) at McMaster University, Hamilton, Ontario, Canada.

The alteration affecting the *Xba*I site at 29.5 m.u. was previously determined to be the result of a 2-bp deletion (Ad5 bp 10594 and 10595) (Thimmappaya et al., 1979). This 2-bp deletion is located 22–23 bp before the initiation site for VAI(A) RNA and prevents its expression, but has no effect on the VAI(G) RNA which initiates 3-bp downstream of VAI(A) (Thimmappaya et al., 1979). Sequencing from the present study showed that the loss of the *Xba*I site at 79.6 m.u. was the result of a 6-bp deletion (Ad5 bp 28597–28602) which affects the coding sequences for

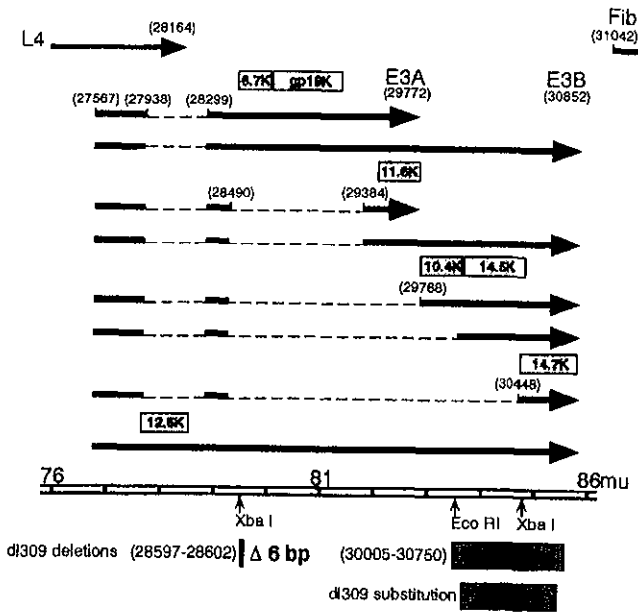

Fig. 1. The structure of the E3 region in wt Ad5 is shown with the locations of the alterations in *dl*309 indicated below. The horizontal arrows indicate the structures of the E3 mRNAs with solid lines representing exons, dashed lines representing introns and arrowheads representing polyadenylation sites. Shaded bars above the arrows represent protein coding sequences. The locations of transcription initiation sites, splice donor and acceptor sites and polyadenylation sites are given in bp. Ad5 sequence positions throughout this article refer to the sequence of wt Ad5 (Chroboczek et al., 1992). To designate Ad5 E3 proteins Ad2 molecular weights were used.

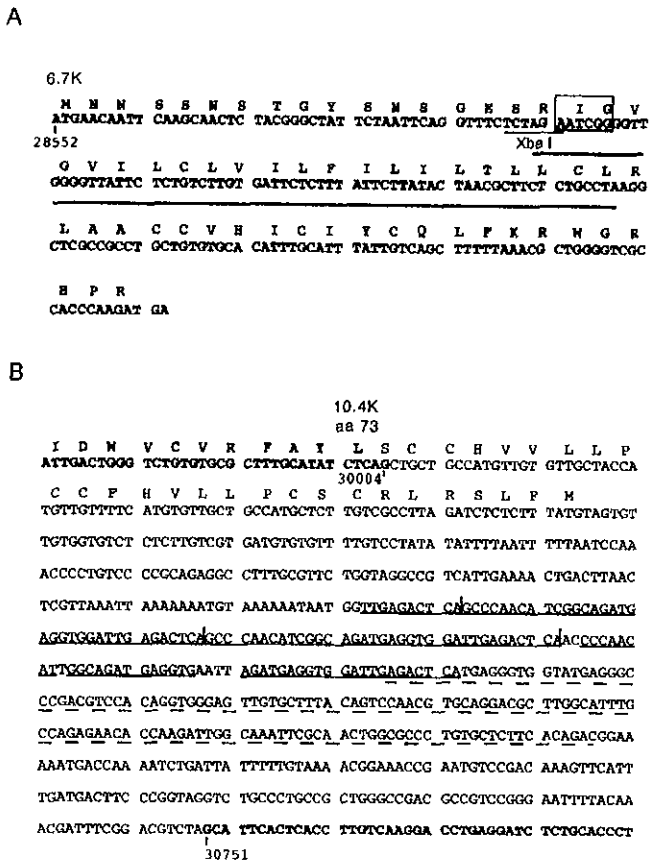

Fig. 2. DNA sequence alterations in the E3 region of *dl*309 are shown. A: the coding sequences for the E3 6.7K protein which span the *Xba*I site at 79.6 m.u. are shown. The loss of the *Xba*I site was due to a 6-bp deletion (Ad5 bp 28597–28602) which removes two amino acids (18 and 19) from the internal hydrophobic region (underlined in bold) which is thought to function in membrane insertion and retention (Wilson-Rawls and Wold, 1993). The 6 bp and two amino acids which are deleted in *dl*309 are boxed. B: the DNA sequence of the deletion/insertion in E3 of *dl*309 is shown. Ad5 sequences flanking the foreign DNA insert are indicated in bold with the last Ad5 nucleotide on each side of the deletion indicated below. The E3 10.4K protein (91 aa in length) is truncated at amino acid 73 but would be predicted to gain 27 amino acids from the insert sequences. Amino acids (single letter code) for the E3 10.4K protein 5′ to the deletion are indicated in bold. The 140-bp segment homologous to the chinook salmon genomic clone of prolactin is indicated with a dashed line. Complete and partial repeat sequences of 35-bp are underlined, and vertical lines indicate the start and end of complete repeats.

the E3 6.7K protein (Fig. 1 and Fig. 2A). 6.7K is a type III integral membrane protein that is retained in the endoplasmic reticulum (Wilson-Rawls and Wold, 1993). The 6-bp deletion removes two amino acids (18 and 19) from the internal hydrophobic region of the protein which is thought to function in membrane insertion and retention (Wilson-Rawls and Wold, 1993). Although 6.7K was

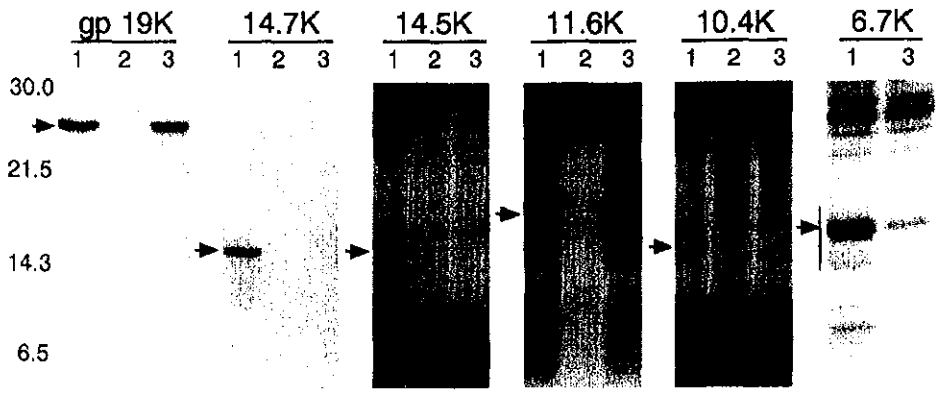

Fig. 3. Immunoprecipitation of E3 proteins from *dl*309-infected A549 cells. The presence of the E3 proteins gp19K, 14.7K, 14.5K, 11.6K, 10.4K and 6.7K was determined for *dl*309 by immunoprecipitation with appropriate antisera (gift of A. E. Tollefson and W. S. M. Wold). A549 cells were infected at an m.o.i. of 50 with wt Ad5, *dl*70-4 which contains a 2.7-kb E3 deletion which disrupts or deletes the coding sequences for all E3 proteins (Bett et al., 1993), or *dl*309 for 7 h and then labelled with 50 μCi each of [^{35}S]methionine and [^{35}S]cystine from 7 to 11 h or infected for 22 h and labelled from 22 to 26 h (to prepare cell extracts for immunoprecipitation of 11.6K only). Cell extracts were then prepared, immunoprecipitated with appropriate antiserum and samples separated by SDS–PAGE on an 18.0% gel after which the gel was dried and bands visualized by autoradiography. Immunoprecipitated extracts obtained from wt Ad5, *dl*70-4 and *dl*309-infected cells were run in lanes marked 1, 2, and 3, respectively, using antisera to the protein indicated above the lanes. Arrows indicate protein bands and molecular weight markers are shown on the left in thousands. The autoradiograms for gp19K and 14.7K represent 6-h exposures while those for 14.5K, 11.6K, 10.4K and 6.7K represent 120-h exposures.

expressed by *dl*309 (Fig. 3) only one of the three 15–16K glycosylated forms found in a wt infection (Wilson-Rawls and Wold, 1993) was detected in *dl*309 infected cell extracts.

The location and sequence of the deletion/insertion in *dl*309 are shown in Figs. 1 and 2B, respectively. The deletion was found to extend from Ad5 bp 30005 to 30750 inclusive, a deletion of 746-bp. Substituted for the deletion is an insertion of 642-bp of non-viral DNA. This alteration disrupts the coding sequences for the E3 10.4K, 14.5K and 14.7K proteins (Cladaras and Wold, 1985). The E3 14.7K protein (Tollefson and Wold, 1988) has been found to protect Ad infected cells from lysis by tumour necrosis factor (Gooding et al., 1988; Gooding et al., 1990; Horton et al., 1991). The 10.4K (Tollefson et al., 1990a) and 14.5K (Tollefson et al., 1990b) proteins form a complex (Tollefson et al., 1991) and have been found to protect cells from lysis by tumour necrosis factor (Gooding et al., 1991) as well as to down-regulate the expression of epidermal growth factor receptor (Carlin et al., 1989; Tollefson et al., 1991) in adenovirus infected cells. Previous studies have shown that the 14.7K protein is not detected in cells infected with *sub*304 (Tollefson and Wold, 1988), the mutant used to derive *dl*309 as described above, in agreement with our sequence (Fig. 1), and expression data (Fig. 3). The 14.5K protein is also not detected in *dl*309 infected cells (Fig. 3). From our sequence

data, the 10.4K protein, normally 91 amino acids long, is predicted to be a fusion protein with the last 18 amino acids removed and 27 amino acids added from the substituted sequences (Fig. 2B). 10.4K could not be detected in *dl*309 infected cells (Fig. 3) with the antisera used in this study. The serum used was raised against a peptide corresponding to aa 68–80 of Ad2 10.4K part of which is deleted in *dl*309. Other E3 proteins that are unaffected by the alterations in the *dl*309 genome, such as gp19K (Persson et al., 1980) and 11.6K (Wold et al., 1984), were detected in *dl*309 infected cells (Fig. 3). The 12.5K protein (Hawkins and Wold, 1992) is also expressed by *dl*309 (W.S.M. Wold, personal communication).

In the study in which *sub*304 was derived, 3 other mutants were obtained with non-viral DNA inserts. When insert sequences in two of these mutants, *sub*305 and *sub*307, were used in reassociation experiments with uninfected HeLa cell DNA, the results suggested that the heterologous DNA was of human origin (Jones and Shenk, 1978), although the percent reassociation was not as high as expected. When the 642-bp of foreign DNA substituted in the E3 region of *dl*309 were used in a homology search of gene bank sequences it was found that a 140-bp stretch was 90% homologous to sequences 3′ to the coding sequences for prolactin in a genomic clone of chinook salmon DNA (Xion et al., 1992) (Fig. 2). The sequence of the human prolactin cDNA (Cooke et al., 1981) is only 28.9% homologous to the sequence of the chinook prolactin clone and the sequences 3′ to the coding region would be expected to be even less well conserved. This suggests that the origin of the foreign DNA in *dl*309 may be that of salmon DNA. The heterologous sequences present in the various insertion mutants obtained in the original study may have originated from the salmon sperm DNA used as a carrier during the transfections performed to select for the mutants (Jones and Shenk, 1978). Previous studies that have analyzed transgenes in cells transformed with exogenous DNA have revealed that in many cases, the transgene DNA is found linked to the carrier DNA used in the transfection (Perucho et al., 1980; Weston et al., 1982). Presumably the exogenous DNA and carrier DNA introduced into the cell using the calcium phosphate technique (Graham and Van der Eb, 1973), are ligated within the cell. The non-viral DNA insert in *dl*309 also contained two direct repeats of 35-bp, flanked by partial repeats, that showed patchy homology (up to 75%) to immunoglobin genes from human and mouse. The presence of sequences in the insert that are partially homologous to human DNA could explain the low level of reassociation obtained using *sub*305 and *sub*307 with HeLa genomic DNA in the original study (Jones and Shenk, 1978), if the inserts in those mutants were also of salmon origin.

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