The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
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).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) 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.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Caroline Schild-Poulter**
DEPARTMENT:	**Robarts Research**
ADDRESS:	**100, Perth Drive**
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EMERGENCY PHONE NUMBER(S):	**519-476-6495**
EMAIL:	**cschild-poulter@robarts.ca**

Location of experimental work to be carried out :

Building : **RRI** Room(s): **Rm3296**

Building : Room(s):

Building : Room(s):

***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: **CIHR**

GRANT TITLE(S): **Characterization of RanBPM function in apoptosis and cell transformation**

UNDERGRADUATE COURSE NAME(IF APPLICABLE):

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

Name	UWO E-mail Address	Date of Biosafety Training
Elnaz Atabakhsh	**eatabakh@uwo.ca**	**Oct-2011**
Victoria Fell	**vfell@uwo.ca**	**Oct 2012**
Louisa Salemi	**lsalemi@uwo.ca**	**Oct 2011**
Wesley Berube-Janzen	**wberubej@uwo.ca**	**May 2012**
Xu Wang	**xwang287@uwo.ca**	**21-Jan-2011**
Keshini Devakandan	**kdevakan@uwo.ca**	**Oct 2012**

Samantha Hershenfeld	shershe2@uwo.ca	June 2010
Matthew Maitland	mmaitla2@uwo.ca	May 2012
Sarah Hoffer	shoffer2@uwo.ca	

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

Microorganisms such as bacteria (DH5α) are used to amplify plasmid DNA. We do not currently use the yeast (S. cerevisiae) but have stored stocks of it at -80°C.
Mammalian cell lines are employed as our experimental system. We use mammalian cells to express various proteins and mutants of these proteins to evaluate the effect of mutation on specific cellular pathways that we are studying.

Storage of biological agents

1. Bacteria

-The stock bacteria are stored in -80oC freezer.
-The bacteria cultures are stored in the fridge which is only use for bacteria storage.

2. Cells
   - -The stock cells are stored in liquid nitrogen.
   - - The cell cultures are kept in CO2 incubator.
3. Plasmid DNAs are stored in -20oC freezer.

Disposal of biological agents

a. Liquid wastes, e.g. cell culture media and bacteria culture are deactivated either by autoclaving or chemical disinfection.
b. Solid wastes are deactivated by autoclaving
c. Sharps wastes are collected in a rigid, leak proof and puncture resistant container and deactivated by incineration

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

Damage to the cells genetic material (DNA) can lead to the development of cancer if not repaired properly. Cells respond to DNA damage by using a variety of pathways to repair the DNA lesions. If repair cannot be completed, cells commit to pathways leading to cell death by suicide, a process called apoptosis. Disruption of the mechanisms that control the repair of DNA and apoptosis results in genomic instability, the underlying cause of carcinogenesis. Moreover, defects in pathways that lead to apoptosis also lead to resistance of cancer cells to therapeutic agents.

We identified RanBPM as a factor that induces cell death when over-expressed in cancer cells. Moreover, we found that RanBPM was necessary for the activation of cell death pathways induced by DNA damage. We have also determined that RanBPM controls the levels of Bcl-2, a critical regulator of the apoptotic machinery that is over-expressed in many types of cancer. Our recent results suggest that RanBPM inhibits the extracellular regulated kinases (ERK) pathway which is often found over-activated in many cancer types.

The aim of our current research is to elucidate the means through which RanBPM promotes cell death and investigate its potential role in preventing tumorigenesis. In particular, we will characterize the determinants of RanBPM that mediate its ability to induce cell death in response to DNA damage. We will investigate how RanBPM functions to inhibit the ERK pathway and determine whether RanBPM functions as a tumour suppressor to prevent tumour formation.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☒ YES ☐ 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? ☐ YES ☒ 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:
Spills are cleaned up with bleach.

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	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, HB101, BL21)	☒ Yes ☐ No	☒ Yes ☐ No	☐ Yes ☒ No	**2 Lt**	**colleagues**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
S. cerevisiae	☒ Yes ☐ No	☒ Yes ☐ No	☐ Yes ☒ No	**2 Lt**	**Clontech**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 2+ ☐ 3
			s			☐ 1 ☐ 2 ☐ 2+ ☐ 3
			s			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ No	☐ No	☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

Not pathogenic

Murine Stem cell Virus

**Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:*
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: ***DH5α, HB101, are used for cloning and plasmid amplification. We mostly use DH5α, but HB101 are recombination-deficient, thus useful for large plasmid cloning. BL21 are employed for protein expression of any gene under the control of a T7 promoter.***

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ☐ 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:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☐ Yes ☒ No		Not applicable
Rodent	☐ Yes ☒ No		
Non-human primate	☐ Yes ☒ No		
Other (specify)	☐ Yes ☒ No		

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)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☒ Yes ☐ No	**See attachment 1**	**2**	**ATCC**
Rodent	☒ Yes ☐ No	**MEFs**	1	**Non-commercial**
Non-human primate	☐ Yes ☐ No			
Other (specify)	☐ Yes ☐ No			

**Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)*

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

Additional Comments: MEFs - mouse embryonic fibroblasts

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☐ YES ☒ 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/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

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? ☒ YES ☐ NO If **NO**, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
E. coli (DH5 α)	**pSuper.Neo,**	**Oligoengine**	**siRNA**	**No**	**No**	**Nothing – siRNA is not targeting the bacterial genome.**

Please attach a Material Safety Data Sheet or equivalent if available.
****Please attach a plasmid map.***
*****No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
☒ YES, complete table below ☐ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Murine stem cell virus	**pMSCV**	**Clontech**	**Ku, RanBPM**	**Re-introduces gene expression in knockout cell line.**

Please attach a Material Safety Data Sheet or equivalent.

4.3.1 Will virus be replication defective? ☒ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☒ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☒ NO

5.0 Will genetic sequences from the following be involved?

- HIV ☒ NO ☐ YES, specify
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ☒ NO ☐ YES, specify
- SV 40 Large T antigen ☒ NO ☒ YES Phoenix Ampho
- E1A oncogene ☐ NO ☒ YES
- Known oncogenes ☐ NO ☒ YES, specify **HPV**
- Other human or animal pathogen and or their toxins ☒ NO ☐ YES, specify

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? ☐ YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 7.0

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

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☐ YES ☒ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used

7.3 AUS protocol #

7.4 List the location(s) for the animal experimentation and housing.

7.5 Will any of the agents listed in section 4.0 be used in live animals
☐ NO ☐ YES, specify:

7.6 Will the agent(s) be shed by the animal:
☐ YES ☐ NO, please justify:

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☐ NO

8.3 If **YES**, please specify the animal(s) used:

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

8.4 If no live animals are used, please specify the source of the specimens:

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☒ YES ☐ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s) **Cholera toxin**
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

We have obtained permission to order but have not ordered it yet.

9.3 What is the LD_{50} (specify species) of the toxin or hormone :260 μg/kg (mouse)

9.4 How much of the toxin or hormone is handled at one time*? 50μg

9.5 How much of the toxin or hormone is stored*? 1 mg

9.6 Will any biological toxins or hormones be used in live animals? ☐ YES ☒ NO
If **YES**, Please provide details:

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

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ YES ☒ 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 insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

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

10.7 Do you use insects that require a permit from the CFIA permit? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

11.4 What is the form of the plant (seed, seedling, plant, tree...)?

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

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

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☐ YES, country of origin ☒ NO
If **NO**, please proceed to Section 13.0

12.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☐ NO

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

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

13.0 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.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Caroline Schild Poulter ***Date:*** Aug 27, 2012

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
☒ YES, location and date of most recent biosafety inspection: **June, 2012**
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-RRI-0026**

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO

If **YES** please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:

We DO NOT use needles with any biological material.

In case of accidental splash with: bacteria or human cell lines, use emergency shower.

15.3 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.shs.uwo.ca/workplace/workplacehealth.html

An X in the check box indicates you agree with the above statement... ☒

Enter Your Name Caroline Schild Poulter ***Date:*** Aug. 27, 2012

15.4 Additional Comments: _____

16.0 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: Ronald Nosworthy

Date: Sept. 05, 2012

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

Special Conditions of Approval:

Attachment 1

Human cell lines:

Containment level 2: Hela, HEK293, Phoenix Amph (HEK293-derivative).

Containment level 1: IMR-90, MCF-10A, HCT116, IMR-32, MCF-7.

Biotechnology INSTITUTE

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10-16-2007, 07:35 AM #1

Simon Holdaway Posts: n/a

Bacterial Transformation Strains

Bacterial Transformation Strains

There are three major strains of E.coli used in current transformation experiments. All are derived from the original, non-pathogenic K12 strain. Many practitioners of transformation experiments do not fully appreciate the importance of the genotypes of these strains and the impact their choice of strain can have on downstream applications for their DNA.

DH5alpha

The E.coli strain DH5alpha is the most common strain used for transformations in research laboratories. Its full genotype is:

F- endA1 glnV44 thi-1 relA1 gyrA96 deoR nupG lacZdeltaM15 hsdR17

The full details of the DH5alpah genome are given at the end of this article, but the major points of interest are that the strain is endA1 ? which allows for clean DNA preparations from plasmid mini-preps, and that it lacks the alpha portion of the lacZ gene and thus can be used for blue-white screening. DH5alpha is also resistant to nalidixic acid.

HB101

Most of you are familiar with the HB101 strain because it is the E.coli strain used by Bio-Rad for their ubiquitous pGLO transformation kit. The full genotype is:

F- mcrB mrr hsdS20(rB-mB-) recA13 leuB6 ara-14 proA2 lacY1 galK2 xyl-5 mtl-1 rpsL20 glnV44

The strain is a hybrid of E.coli strains K12 and E.coli B and is the best host for pBR322. Biorad uses this strain because it is unable to metabolize arabinose. The GFP gene on the pGLO plasmid is under the control of an arabinose promoter. Adding arabinose turns on the promoter. If the E.coli could metabolize the arabinose sugar then the promoter would eventually turn off as arabinose disappeared from the cell. Thus GFP expression would be lost. HB101 lacks the endA1 genotype, which means plasmid mini-preps from this strain will be lower yield and less ?clean? due to the action of a non-specific DNA endonuclease that HB101 produces, but DH5alpha does not. Interestingly, HB101 is also resistant to streptomycin, so don?t use that antibiotic as a selective marker in your transformation experiments!

JM109

The final ?common? transformation strain is JM109, a strain originally developed for cloning repetitive

DNA. Some transformation educational kits on the market still use this strain. The genotype is:

endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ delta(lac-proAB) e14- [F? traD36 proAB+ lacIq lacZdeltaM15] hsdR17

The important things to know about JM109 are that it is good for cloning and downstream DNA applications (no endonuclease activity). It is nalidixic acid resistant and allows cloning of unmethylated DNA from PCR reactions. It can be used for blue-white screening, unlike HB101, but most scientists prefer the DH5alpha strain

JM109 ? not used

DH5alpha genome explained:

DH5 alpha: F- endA1 glnV44 thi-1 relA1 gyrA96 deoR nupG lacZdeltaM15 hsdR17

F- does not carry the F+ plasmid for conjugation. An important step in ensuring biosafety.

endA1 does not produce Endonuclease I. This allows cleaner plasmid preps since you avoid non-specific digestion of plasmid DNA.

glnV44 suppression of the amber (or UAG) stop codon and replacement with glutamine. This is required for some bacteriophage growth.

Thi-1 requires thiamine

relA1 permits production of RNA without protein synthesis

gyrA96 nalidixic acid resistance ? conferred by a mutation on DNA gyrase

deoR permits uptake of larger sized plasmids

nupG also allows uptake of large plasmids

lacZdeltaM15 deletion of the alpha portion of the beta-galactosidase gene. Allows blue-white screening

hsdR17 allows cloning of unmethylated DNA ? mostly from PCR reactions

HB101 genome explained

F- mcrB mrr hsdS20(rB-mB-) recA13 leuB6 ara-14 proA2 lacY1 galK2 xyl-5 mtl-1 rpsL20 glnV44

The strain is a hybrid of E.coli strains K12 and E.coli B and is the best host for pBR322.

F- does not carry the F+ plasmid for conjugation. An important step in ensuring biosafety since it prevents accidental dissemination of plasmids.

mcrB eliminates restriction of DNA at the sequence R(m)C

mrr eliminates restriction of DNA at the sequence C(m)Ag or G(m)AC

hsdS20(rB-mB-)

recA13 reduces recombination in cloned DNA, UV sensitive because of DNA repair deficiency

leuB6 requires leucine

ara-14 unable to metabolize arabinose

proA2 requires proline

lacY1 deletion of lactose permease (lacY) from lac operon

galK2 unable to metabolize galactose

xyl-5 unable to metabolize xylose

mtl-1 unable to metabolize mannitol

rpsL20 mutation in ribosome, conveying streptomycin resistance

gln44 suppression of the amber (or UAG) stop codon and replacement with glutamine. This is required for some bacteriophage growth.

JM109

endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ delta(lac-proAB) e14- [F? traD36 proAB+ lacIq lacZdeltaM15] hsdR17

endA1 no endonuclease I activity. Allows for cleaner DNA preparations.

glnV44 suppression of amber (UAG) stop codons by insertion of glutamine

thi-1 requires thiamine

relA1 relaxed phenotype which allows mRNA synthesis without protein synthesis

gyrA96 nalidixic acid resistant

recA1 reduces recombination in cloned DNA, UV sensitive because of DNA repair deficiency

mcrB+ no restriction at methylated RmC sequences

delta(lac-proAB)

e14- contains a prophage remnant which contains the mcrA gene

[F? traD36 proAB+ lacIq lacZdeltaM15] carries an F plasmid (for conjugation) carrying these chromosomal genes

hsdR17 required for transformation of unmethylated DNA (from PCR rxns)

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Posting Rules

Conforms to 93/112/EC and ISO 11014-1

SAFETY DATA SHEET

Novagen®

Order Number

Customer Number

1. Identification of the substance/preparation and of the company/undertaking

Product name : BL21(DE3) Glycerol Stock

Catalog # 69387

Supplier : Manufactured by EMD Biosciences, Inc.
441 Charmany Drive
Madison, WI 53719
(608)238-6110
(800)207-0144
FAX: (608)238-1388

P.O. Box 12087
La Jolla, CA 92039-2087
(858)450-5558
(800)854-3417
FAX: (858)453-3552

Synonym Not available.

Emergency telephone number : **Call Chemtrec®**
(800)424-9300 (within U.S.A.)
(703)527-3887 (outside U.S.A.)

2. Composition / information on ingredients

Substance/Preparation : Substance

Chemical name*	CAS No.	EC Number	Symbol	R-Phrases
BL21(DE3) Glycerol Stock		Not available.	-	-

3. Hazards identification

Physical/chemical hazards : Not applicable.

Human health hazards : No specific hazard.

4. First-aid measures

First-Aid measures

Inhalation : If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

Ingestion : Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

Skin Contact : In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.

Eye Contact : Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.

Aggravating conditions : Repeated or prolonged exposure is not known to aggravate medical condition.

5. Fire-fighting measures

Flammability of the Product : May be combustible at high temperature.

Extinguishing Media

Suitable : SMALL FIRE: Use DRY chemical powder.
LARGE FIRE: Use water spray, fog or foam. Do not use water jet.

Special fire-fighting procedures : Fire fighters should wear positive pressure self-contained breathing apparatus (SCBA) and full turnout gear.

Protection of fire-fighters : Be sure to use an approved/certified respirator or equivalent.

6. Accidental release measures

Personal precautions : Splash goggles. Full suit. Boots. Gloves. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Small Spill and Leak : Absorb with an inert material and put the spilled material in an appropriate waste disposal.

Large Spill and Leak : Absorb with an inert material and put the spilled material in an appropriate waste disposal.

7. Handling and storage

Handling : Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe gas/fumes/ vapor/spray.

Storage : Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above -70°C (-94°F).

Packaging materials

Recommended use : Use original container.

8. Exposure controls/personal protection

Engineering measures : Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

Hygiene measures : Wash hands after handling compounds and before eating, smoking, using lavatory, and at the end of day.

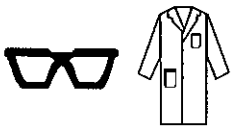
Ingredient Name	Occupational Exposure Limits
BL21(DE3) Glycerol Stock	Not available.

Personal protective equipment

Skin and body : Lab coat.

Eyes : Safety glasses.

Protective Clothing (Pictograms) :



9. Physical and chemical properties

Physical state : Liquid.

Color : Not available.

Molecular Weight : Not available.

Solubility : Not available.

Flash point : Not available.

Explosive properties : Risks of explosion of the product in presence of mechanical impact: Not available.
Risks of explosion of the product in presence of static discharge: Not available.

10. Stability and reactivity

Stability : The product is stable.

Conditions to avoid : Not available.

11. Toxicological information

RTECS # : **Not available.**

Local effects

Skin irritation : Not available.

Acute toxicity : LD50: Not available.
LC50: Not available.

Chronic toxicity : Repeated or prolonged exposure is not known to aggravate medical condition.

Other Toxic Effects on Humans : Not available.
No specific information is available in our database regarding the other toxic effects of this material for humans.

Not available.

To the best of our knowledge, the toxicological properties of this product have not been thoroughly investigated.

Carcinogenic effects : Not available.

Mutagenic effects	:	Not available.
Reproduction toxicity	:	Not available.
Teratogenic effects	:	Not available.

12. Ecological information

Ecotoxicity	:	Not available.
Toxicity of the Products of Biodegradation	:	Not available.

13. Disposal considerations

Methods of disposal; Waste of residues; Contaminated packaging	:	Waste must be disposed of in accordance with federal, state and local environmental control regulations.

14. Transport information

International transport regulations

Land - Road/Railway

ADR/RID Class	:	Not controlled under ADR (Europe).

Sea

IMDG Class	:	Not controlled under IMDG.

Air

IATA-DGR Class	:	Not controlled under IATA.
Special Provisions for Transport		Not applicable.

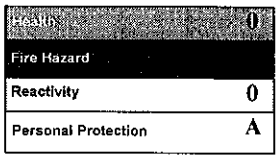
15. Regulatory information

EU Regulations

Risk Phrases	:	This product is not classified according to the EU regulations.
U.S. Federal Regulations	:	TSCA: No products were found. SARA 302/304/311/312 extremely hazardous substances: No products were found. SARA 302/304 emergency planning and notification: No products were found. SARA 302/304/311/312 hazardous chemicals: No products were found. SARA 311/312 MSDS distribution - chemical inventory - hazard identification: No products were found. SARA 313 toxic chemical notification and release reporting: No products were found. Clean Water Act (CWA) 307: No products were found. Clean Water Act (CWA) 311: No products were found. Clean air act (CAA) 112 accidental release prevention: No products were found. Clean air act (CAA) 112 regulated flammable substances: No products were found. Clean air act (CAA) 112 regulated toxic substances: No products were found.
HCS Classification	:	Not controlled under the HCS (United States).
State Regulations	:	
WHMIS (Canada)	:	Not controlled under WHMIS (Canada). No products were found.

16. Other information

Hazardous Material Information System (U.S.A.)



National Fire Protection Association (U.S.A.)

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. **This product is intended for research use only.***

Certificate of Analysis

In the interest of conserving resources, we are no longer shipping manuals with products. Please visit **www.clontech.com/manuals** to obtain an electronic version.

PRODUCT: Y2HGold Yeast Strain

CATALOG No. 630498

LOT NUMBER
Specified on product label.

STORAGE CONDITIONS
–70°C

SHELF LIFE
1 year from date of receipt under proper storage conditions

SHIPPING CONDITIONS
Dry ice (–70°C)

DESCRIPTION
The *Saccharomyces cerevisiae* strain Y2HGold is designed for use with Clontech's Matchmaker™ Gold Yeast Two-Hybrid System. Y2HGold includes the sensitive Aureobasidin A antibiotic resistance gene as one of four reporters—providing exceptionally stringent yeast two-hybrid (Y2H) screening. This strain contains distinct *ADE2, HIS3, MEL1,* and *AUR1-C* reporter constructs that are only expressed in the presence of GAL4-based protein interactions. Y2HGold is extremely effective in minimizing false positive protein interactions and background during a typical GAL4-based two-hybrid screen.

PACKAGE CONTENTS
- 0.5 ml *Saccharomyces cerevisiae* Y2HGold
- Matchmaker Gold Yeast Two-Hybrid System User Manual (PT4084-1)

FOR RESEARCH USE ONLY

QUALITY CONTROL DATA

- The nutritional requirements (i.e., auxotrophic markers) of Y2HGold Yeast Strain were verified by streaking samples onto six types of SD minimal media: SD/-Ade, SD/-His, SD/-Leu, SD/-Trp, SD/-Ura, and SD/-Met.
- Reporter function. The function of the *ADE2, HIS3, MEL1* and *AUR1-C* reporters in Y2HGold Yeast Strain were verified using positive and negative control plasmids and plating on minimal media lacking adenine and histidine, and containing X-alpha-Gal and Aureobasidin A.

APPROVED BY: _____

(PA993388)

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Technical Support (US)
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Attachment 1

Cell Line(s)

Human cell lines:

Containment level 2: Hela, HEK293, Phoenix Amph (HEK293-derivative).

Containment level 1: IMR-90, MCF-10A, HCT116, IMR-32, MCF-7.

Cell Biology

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

Designations:	HeLa
Depositors:	WF Scherer
Biosafety Level:	2 [CELLS CONTAIN PAPOVAVIRUS]
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
Reverse Transcript:	negative
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:	

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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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

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.

Comments: 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]

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%.

Propagation: **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:

Information

Home
About the NGVB
Policies and Procedures
What's New
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Gene Therapy Data Maps
Archiving Services
Insertion Site Analysis
Pharm/Tox Resources
Clinical Trial Testing
Reagent Repository
Educational Resources
My NGVB

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user name:

password:

Login

Get user name and password

Can't remember your password?

Reagents | Cart | Checkout

Reagent

Add to Cart

Reagent Information	
Name	AmphoPhoenix
Reagent Type	Cell Line
Vector Classifications	Retrovirus
Keywords	
Cell Line Origin	HEK293T
Short Description	
Long Description	AmphoPhoenix is a vector packaging cell line containing the retroviral gag/pol genes. The vector particles are pseudotyped with the 4070A Amphotropic Virus envelope glycoprotein. Insertion of a retroviral vector construct will lead to vector production, either through transient transfection methods or through establishing a stable cell line.
Vector Map	
Sequence	
Grade	Suitable for use in Phase I clinical trials
Depositor	The AmphoPhoenix cell line was created at Stanford University in the laboratory of Dr. Garry Nolan.
References	www.stanford.edu/group/nolan
Biosafety	This cell line could theoretically develop recombinations leading to the development of replication competent retroviruses, although it has failed to do so in extensive testing and clinical scale productions. The Indiana Unversity Vector Production Facility works with this line under BL-2 containment but the level of containment required of any cell line or vector is at the discretion of the investigator's Institutional Biosafety Committee that may require a different level of containment.
Comments	As only a limited number of certified vials are available, the material will be distributed only to those investigators conducting federally funded development of clinical trial material.Grant number and specific aims should be provided to the Coordinating Center with cell line request. Individuals seeking this line for reserch purposes should obtain the cells through the ATCC (www.atcc.org).
Storage	
Availability	

The NGVB is supported by the NIH'sNational Heart, Lung, and Blood Institutethrough a grant to the Indiana University School of MedicineDepartment of Medical and Molecular Genetics

Cell Biology

ATCC® Number:	**CCL-247™**	Order this Item	Price:	**$256.00**

Designations:	HCT 116
Depositors:	MG Brattain
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** colon **Disease:** colorectal carcinoma
Cellular Products:	carcinoembryonic antigen (CEA) 1 ng per 10 exp6 cells per 10 days; 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 (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Tumorigenic:	Yes
Reverse Transcript:	negative
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 7,10 D13S317: 10,12 D16S539: 11,13 D5S818: 10,11 D7S820: 11,12 THO1: 8,9 TPOX: 8,9 vWA: 17,22
Cytogenetic Analysis:	The stemline chromosome number is near diploid with the modal number at 45 (62%) and polyploids occurring at 6.8%. The markers 10q+ and t(?8p;18q) are present in all metaphases and t(9q;?16p-), in 80% of the cells karyotyped. N16 is monosomic in the presence of, but disomic in the absence of t (9q;?16p-). N10 and N18 are monosomic and other chromosomes from those mentioned above are disomic. Q-band observations revealed the presence of the Y chromosome, but not in all cells (50% of cells lacked the Y in G-band karyotypes).

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Isoenzymes:	AK-1, 1 ES-D, 1-2 G6PD, B GLO-I, 1 PGM1, 1 PGM3, 1
Age:	adult
Gender:	male
Comments:	The cells are positive for keratin by immunoperoxidase staining. HCT 116 cells are positive for transforming growth factor beta 1 (TGF beta 1) and beta 2 (TGF beta 2) expression. This line has a mutation in codon 13 of the ras protooncogene, and can be used as a positive control for PCR assays of mutation in this codon.
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%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Growth Conditions:** Growth and plating efficiency are enhanced by using a feeder layer of murine fibroblasts.
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 1:3 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

Product Information Sheet for ATCC® CRL-10317™

Cell Line Designation: MCF 10A
ATCC® Catalog No. CRL-10317™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: mammary gland; breast; fibrocystic disease;
Age: 36 years
Gender: female
Morphology: epithelial
Growth properties: adherent
Tumorigenic: no; The cells were not tumorigenic in immunosuppressed mice, but did form colonies in semisolid medium.
DNA profile (STR analysis):

Amelogenin: X
CSF1PO: 10,12
D13S317: 8,9
D16S539: 11,12
D5S818: 10,13
D7S820: 10,11
TH01: 8,9.3
TPOX: 9,11
vWA: 15,17

Depositors: Michigan Cancer Foundation
Comments: The MCF 10A cell line is a non-tumorigenic epithelial cell line. The line was produced by long term culture in serum free medium with low Ca++ concentration.
MCF 10A was derived from adherent cells in the population. Cells derived from a floating population are available (see MCF 10F, ATCC CRL-10318). The cells are positive for epithelial sialomucins, cytokeratins and milk fat globule antigen.
They exhibit three dimensional growth in collagen, and form domes in confluent cultures. Thus far, the cells have shown no signs of terminal differentiation or senescence.
The line is responsive to insulin, glucocorticoids, cholera enterotoxin, and epidermal growth factor (EGF). By electron microscopy the cells display characteristics of luminal ductal cells but not of myoepithelial cells. They also express breast specific antigens as detected by positive reaction with MFA-Breast and MC-5 monoclonal antibodies. The calcium content of the medium exerts a strong effect on the morphology of the cells.
Karyotype: aneuploid human female; 48, XX, +8, +16, 3p-, 6p+, 9p+

Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. Cell lines and hybridomas deposited for patent purposes are not always screened for contamination, antibody production or characterized by the ATCC. Release of a culture, the use of which may be claimed in a patent, from the ATCC during the effective term of any such patent is not meant to carry with it, and does not grant any license, express or implied, under any patent, or the right to use a culture in any process described in a patent.
The above culture was deposited in the ATCC in connection with a patent application. Copies of U.S. Patents may be obtained from the Commissioner of Patents, U.S. Patent and Trademark Office, Box 9, Washington, D.C. 20231.
This material is cited in a U.S and/or other Patent and may not be used to infringe the patent claims

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 x *g* for 5 to 10 minutes.

4. Resuspend the cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x *g* for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for a 75 cm^2 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 Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.05% (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 0.05% Trypsin-0.53 mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 10 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 3.0 ml solution of 0.1% soybean trypsin inhibitor.

5. Transfer cell suspension to a centrifuge tube and spin at approximately 125 x *g* for 5 to10 minutes.

6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
   Subcultivation Ratio: 1:3 to 1:4

7. Place culture vessels in incubators at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Medium Renewal

Two to three times weekly

Complete Growth Medium

The base medium for this cell line (MEBM) along with the additives can be obtained from Lonza/Clonetics Corporation as a kit: MEGM, Kit Catalog No. CC-3150. ATCC does not use the GA-1000 (gentamycin-amphotericin B mix) provided with the kit.
To make the complete growth medium, you will need to add the following component (sold separately):

- 100 ng/ml cholera toxin

Note: Do not filter complete medium
This medium is formulated for use with a 5% CO_2 in air atmosphere.

Cryoprotectant Medium

Complete growth medium described above supplemented with 7.5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at **www.atcc.org**)

Soule H and McGrath CM. **Immortal human mammary epithelial cell lines**. U.S. Pat. 5,026,637 dated June 25, 1991

Pauley RJ et al. Immortal **human mammary epithelial cell sublines**. U.S. Pat. 5,206,165 dated Apr. 27, 1993

Soule HD and McGrath CM. **A simplified method for passage and long-term growth of human mammary epithelial cells**. In Vitro 22: 6-12, 1986 PubMed: 86111509

Soule HD et al. **Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10.** Cancer Res. 50: 6075-6086, 1990 PubMed: 90367052

Tait L et al. **Ultrastructural and immunocytochemical characterization of an immortalized human breast epithelial cell line, MCF-10.** Cancer Res. 50: 6087-6094, 1990 PubMed: 90367053

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

01/12

Cell Line Designation: IMR-90
ATCC® Catalog No. CCL-186

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: Lung
Age: 16 weeks gestation; fetus.
Gender: Female
Ethnicity: Caucasian
Morphology: Fibroblast
Growth properties: adherent
VirusSuscept: poliovirus 1, 2, 3; herpes simplex 1, 2; varicella zoster; vesicular stomatitis; cytomegalovirus; vaccinia
Reverse Transcriptase: negative
DNA profile (STR nalysis)

Amelogenin: X
CSF1PO: 11,13
D13S317: 11,13
D16S539: 10,13
D5S818: 12,13
D7S820: 9,12
TH01: 8,9.3
TPOX: 8,9
vWA: 16,19

Depositor: W.W. Nichols
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. 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.
Karyotype: normal human female; diploid; stable
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Biosafety Level: 1

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens. ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming et al., 1995) the ATCC manual on quality control (Hay et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and in the U.S. Government Publication, ***Biosafety in Microbiological and Biomedical Laboratories*** , 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

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Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a **37°C** water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.

4. Transfer the cells to an appropriate size vessel. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be*

placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 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 0.25% (w/v) Trypsin-0.53mM 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.
   Subcultivation Ratio: 1:2 to 1:8.

6. Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in ***Culture of Animal Cells, a manual of Basic Technique*** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

Two to three times weekly

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%

This medium is formulated for use with a 5% CO_2 in air atmosphere.
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020 (500ml) and ATCC Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at www.atcc.org)

Nichols WW et al. **Characterization of a new human diploid cell strain, IMR-90.** Science 196: 60-63, 1977 PubMed: 77127332

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: 96347553

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: 96215295

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture.** J. Tissue Culture Methods 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

Centers for Disease Control (1993), **Biosafety in Microbiological and Biomedical Laboratories** Human Health Service Publication No. (CDC) 93-8395. U.S. Dept. of Health and Human Services; 3rd Edition U.S. Government Printing Office Washington D.C.

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Product Information Sheet for ATCC® HTB-22™

Cell Line Designation: MCF-7
ATCC® Catalog No. HTB-22™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: mammary gland; breast adenocarcinoma; derived from metastatic site: pleural effusion
Age: 69 years
Gender: female
Ethnicity: Caucasian
Morphology: epithelial
Doubling time: about 29 hours
Growth Properties: adherent
Oncogene: wnt7h +
Antigens Expressed: Blood Type O; Rh+
Products: insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5
DNA profile (STR analysis)

Amelogenin: X
CSF1PO: 10
D13S317: 11
D16S539: 11,12
D5S818: 11,12
D7S820: 8,9
TH01: 6
TPOX: 9,12
vWA: 14,15

Depositor: C.M. McGrath
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.
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.
Karyology: 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.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Purified DNA from this line is available as ATCC® HTB-22D™ (10µg)
Total RNA from this line is available as ATCC® HTB-22R™ (100µg)

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

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Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*
3. It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at

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Or contact your local distributor.

approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.

4. Transfer the cell pellet to an appropriate size vessel. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Note: Present batches of MCF7 cells are exhibiting the following growth pattern:
The cells usually attach as three-dimensional clusters and eventually grow to a 80-90% confluent monolayer. However, we are finding that most of the clusters remain in suspension until after the 2nd subculture.
After first subculture all the cells will not attach. There will be clusters in suspension. Break up the clusters the best you can by gently pipetting with a small bore pipette (5 ml or smaller). After a few days incubation, the cells should reattach as three-dimensional islands (there will be some clusters that do not reattach). Growth will eventually spread out from the islands and the culture should, after the second subculture, flatten and become 70-80% confluent.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm2 flask; 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 with 5 to 10 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. Transfer the cell suspension to the centrifuge tube with the medium and cells from Step #1 and spin at approximately 125 xg for 5 to10 minutes. Discard the supernatnant.

6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. Maintain cultures at a cell concentration between 2x10(4) and 2 x 10(5) cells/cm2.
   Subcultivation Ratio: 1:3 to 1:6.

7. Place culture vessels in incubators at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Medium Renewal

Two to three times weekly

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:

- 0.01mg/ml bovine insulin
- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO_2 in air atmosphere.

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ATCC tested fetal bovine serum is available as ATCC® Catalog No. 30-2020 (500ml) or ATCC® Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog at www.atcc.org)

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: 86044518

Takahashi K and Suzuki K. **Association of insulin-like growth-factor-I-induced DNA synthesis with phosphorylation and nuclear exclusion of p53 in human breast cancer MCF-7** cells. Int. J. Cancer 55: 453-458, 1993 PubMed: 93388025

Brandes LJ and Hermonat MW. **Receptor status and subsequent sensitivity of subclones of MCF-7 human breast cancer cells surviving exposure to diethylstilbestrol**. Cancer Res. 43: 2831-2835, 1983 PubMed: 83206536

Lan MS et al. **Polypeptide core of a human pancreatic tumor mucin antigen**. Cancer Res. 50: 2997-3001, 1990 PubMed: 90242270

Pratt SE and Pollak MN. **Estrogen and antiestrogen modulation of MCF7 human breast cancer cell proliferation is associated with specific alterations in accumulation of insulin-like growth factor-binding proteins in conditioned media**. Cancer Res. 53: 5193-5198, 1993 PubMed: 94036798

Huguet EL et al. **Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue**. Cancer Res. 54: 2615-2621, 1994 PubMed: 94221588

Soule HD et al. **A human cell line from a pleural effusion derived from a breast carcinoma**. J. Natl. Cancer Inst. 51: 1409-1416, 1973 PubMed: 74054239

Bellet D et al. **Malignant transformation of nontrophoblastic cells is associated with the expression of chorionic gonadotropin beta genes normally transcribed in trophoblastic cells**. Cancer Res. 57: 516-523, 1997 PubMed: 97164677

Littlewood-Evans AJ et al. **The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma**. Cancer Res. 57: 5386-5390, 1997 PubMed: 98053913

Komarova EA et al. **Intracellular localization of p53 tumor suppressor protein in gamma-irradiated cells is cell cycle regulated and determined by the nucleus**. Cancer Res. 57: 5217-5220, 1997 PubMed: 98053886

van Dijk MA et al. **A functional assay in yeas for the human estrogen receptor displays wild-type and variant estrogen receptor messenger RNAs present in breast carcinoma**. Cancer Res. 57: 3478-3485, 1997 PubMed: 97413630

Landers JE et al. **Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein**. Cancer Res. 57: 3562-3568, 1997 PubMed: 97413643

Umekita Y et al. **Human prostate tumor growth in athymic mice: inhibition by androgens and stimulation by finasteride**. Proc. Natl. Acad. Sci. USA 93: 11802-11807, 1996 PubMed: 97030277

Zamora-Leon SP et al. **Expression of the fructose transporter GLUT5 in human breast cancer**. Proc. Natl. Acad. Sci. USA 93: 1847-1852, 1996 PubMed: 96312501

Geiger T et al. **Antitumor activity of a PKC-alpha antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice.** Anti-Cancer Drug Des. 13: 35-45, 1998 PubMed: 98134516

Jang SI et al. **Activator protein 1 activity is involved in the regulation of the cell type-specific expression from the proximal promoter of the human profilaggrin gene**. J. Biol. Chem. 271: 24105-24114, 1996 PubMed: 96394543

Lee JH et al. **The proximal promoter of the human transglutaminase 3 gene**. J. Biol. Chem. 271: 4561-4568, 1996 PubMed: 96224044

Chang K and 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: 96133892

Zhu X et al. **Cell cycle-dependent modulation of telomerase activity in tumor cells**. Proc. Natl. Acad. Sci. USA 93: 6091-6095, 1996 PubMed: 96234095

Bacus SS et al. **Differentiation of cultured human breast cancer cells (AU-565 and MCF-7) associated with loss of cell surface HER-2/neu antigen.** Mol. Carcinog. 3: 350-362, 1990 PubMed: 91119659

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

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Section 4

pSUPER RNAi System™

VECTOR: pSUPER.neo
CATALOG#: VEC-PBS-0003/0004

Length: 4699 bp

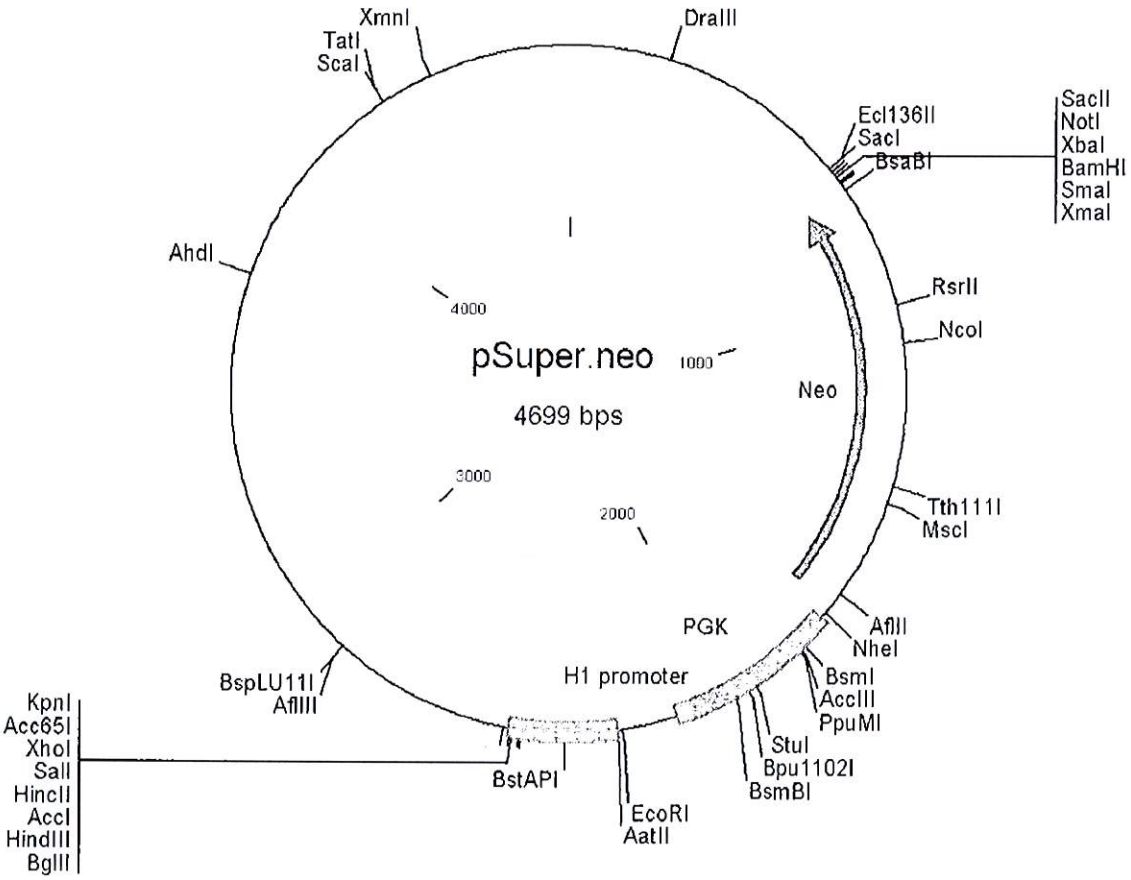
Key Sites

BglII: 2451
HindIII: 2457
EcoRI: 2230
SalI: 2472
XhoI: 2478

Vector Features

f1(+) origin: 135-441
PGK promoter: 2110-1712
Neo ORF: 1684-715
H1 promoter: 2235-2483
Ampicillin resistance ORF: 3714-4193

T7 primer binding site (AATACGACTCACTATAG): 627-643
T3 primer binding site (CTTTAGTGAGGGTTAAT): 2512-2528
M13(-20) primer binding site (GTAAAACGACGGCCAGT): 600-616
M13 reverse primer binding site (CATGGTCATAGCTGTT): 2546-2561



Clontech

MSCV Retroviral Expression System

You may also be interested in these Clontech products:

Lenti-X™ Vector Overview
Fluorescent Lentiviral Expression Vectors
Viral Vectors for Fluorescent Protein Delivery
Fluorescent Retroviral Vectors
Lentiviral Inducible Expression Systems
Lenti-X™ Expression Systems
Lenti-X™ HT Packaging Systems

The MSCV (Murine Stem Cell Virus) Retroviral Expression System contains vectors that are optimized for introducing and expressing target genes in pluripotent cell lines, including murine or human hematopoietic, embryonic stem (ES), and embryonal carcinoma (EC) cells. They can also be used effectively with any mammalian cell line (1–3). This highly efficient system is ideal for analyzing gene function in development, embryogenesis, or immune response, in both cell culture and transgenic assays.

Designed for Difficult-to-Infect Cells

The MSCV System contains three vectors: pMSCVneo, pMSCVhyg, and pMSCVpuro. These vectors contain a specially designed long terminal repeat (LTR) from the murine stem cell PCMV virus that allows you to work with hard-to-transduce cell lines. This LTR differs from the MMLV LTR by several point mutations and a deletion that together enhance transcriptional activation and prevent transcriptional suppression in ES and EC cells. As a result, the PCMV LTR drives high-level, constitutive expression of the target gene in stem cells or other mammalian cell lines. The MSCV System includes the RetroPack™ PT67 Packaging Cell Line, which produces high-titer virus able to infect a broad range of mammalian host cells.

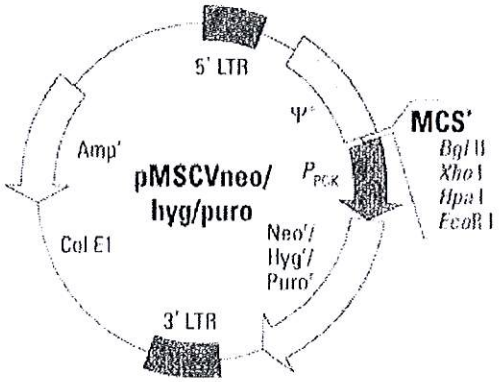


Figure 1. pMSCV Vectors map.

References

1. Hawley, R. G., *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:10297–10302.
2. Keller, G., *et al.* (1998) *Blood* 92 877–887.
3. Hawley, R. G., *et al.* (1994) *Gene Ther.* 1:136–138.

Components

pMSCVneo Vector

Storage Conditions

–196°C for RetroPack PT67 Cell Line

Western
UNIVERSITY · CANADA

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Cholera toxin
Proposed Use Dose:	50 µg
Proposed Storage Dose:	1000 µg
LD_{50} (species):	260 µg

Calculation:

260 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 13000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 1300

Comments/Recommendations:

Toxin

CALBIOCHEM®

SAFETY DATA SHEET (MSDS)

Date of Preparation or Revision: September 20, 2001

IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND COMPANY:

Catalog Number:	227043
Product Name:	Cholera Toxin B Subunit, Saporin Conjugate
Supplier:	CALBIOCHEM®
	P.O. Box 12087
	La Jolla, CA 92039-2087
Telephone:	(858) 450-5558
	(800) 854-3417
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Emergency Contact:	CHEMTREC®
	(800) 424-9300 (within U.S.A.)
	(703) 527-3887 (outside U.S.A.)

COMPOSITION/INFORMATION ON INGREDIENTS:

Chemical Name:	Cholera Toxin B Subunit, Saporin Conjugate
Hazardous Ingredient:	Cholera Toxin

HAZARDOUS IDENTIFICATION:

May be harmful by ingestion or absorption through skin. Material may cause irritation to the eyes, skin, mucous membranes, and the upper respiratory tract. The B subunit of Cholera Toxin is non-toxic in the absence of the A subunit. Due to the possibility of slight contamination of this product with intact toxin or B subunit, it is considered potentially toxic. The intact toxin is a natural, highly toxic compound. Cholera toxin produces a massive outpouring of small intestinal secretions. This results in a net loss of body water and electrolytes. Material is an activator of adenylate cyclase. *In vivo*, Cholera toxin causes edema and erythema.

FIRST AID MEASURES:

In cases of skin contact, wash immediately with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes and wash before wearing. Consult a physician.
In cases of eye contact, flush immediately with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Consult a physician.
In cases of inhalation, remove to fresh air and monitor breathing. If breathing becomes difficult, give oxygen and consult a physician. If breathing stops, give artificial respiration and consult a physician.
In cases of ingestion, wash mouth out with water and consult the local poison center and a physician.

FIRE-FIGHTING MEASURE:

Extinguishing Media: Dry chemical powder, carbon dioxide, water spray, alcohol or polymer foam.
Special Fire Fighting Procedures: Wear self-contained breathing apparatus and full protective clothing to prevent contact with skin and eyes.
Unusual Fire and Explosive Hazards: Upon thermal decomposition, it may **EMIT TOXIC GASES**.

ACCIDENTAL RELEASE MEASURES:

Steps to be Taken if Material is Released or Spilled: Evacuate area. Wear a self-contained breathing apparatus, rubber boots, and heavy rubber gloves. Sweep up and place in a suitable container. Avoid raising dust. Hold for appropriate disposal. Wash spill site and ventilate area after material pickup is complete.

HANDLING AND STORAGE:

Store in a tightly closed container in a +4°C refrigerator. Material is harmful and an irritant. Avoid contact with material. Avoid prolonged or repeated exposure. Lab should be equipped with a safety shower and an eye wash station. Wash thoroughly after handling material.

EXPOSURE CONTROLS/PERSONAL PROTECTION:

Protective Equipment: Wear suitable protective clothing, such as chemical resistant gloves and chemical safety goggles.

Respiratory Protection and Ventilation: Use a NIOSH/MSHA approved respirator. Handle in a chemical fume hood with adequate ventilation.

PHYSICAL AND CHEMICAL PROPERTIES:

Appearance: Solid lyophilized from 20 mM sodium phosphate buffer, pH 7.5.
Flash Point: N/A
Solubility: Reconstitute with 100 µl of sterile distilled water.

STABILITY AND REACTIVITY:

Stability: Stable under normal handling procedures.
Incompatibilities and Conditions to Avoid: N/A
Hazardous Combustion or Decomposition Products: Upon thermal decomposition, may emit toxic gases including carbon monoxide and carbon dioxide.

TOXICOLOGICAL INFORMATION:

RTECS Number: LF3100000 (Cholera Toxin)

Toxicity Data and References:

Ivn; rbt; LDLo: 100 µg/kg 1981. *Toxicon.* **19,** 701.
Ivn; mky; LDLo: 10 µg/kg Changes in sense organs and food intake; dyspnea 1980. *Toxicon.* **18,** 309.
Ivn; mus; LD_{50}: 260 µg/kg 1972. *Immunological Communications.* **1,** 223.

Reproductive Effects and References:

Ivn; mus; Dose: 40 µg/kg Post-implantation mortality 1975. *Journal of Reproduction and Fertility.* **45,** 315.
Ivn; mus; Dose: 8 µg/kg Post-implantation mortality; abortion 1972. *Immunological Communications.* **1,** 223.

Genetic Data and References:

Oth; mus; Dose: 10 µg/l Unscheduled DNA synthesis 1987. *Carcinogenesis (London).* **8,** 377.

Refer to the Registry of Toxic Effects of Chemical Substances (RTECS) for a definition of abbreviations used in the above text and for additional information. This report contains only selected information from the RTECS.

ECOLOGICAL INFORMATION:

No information available.

DISPOSAL CONSIDERATIONS:

Country, federal, state, and local regulations are varied and change frequently. For this reason, we recommend that you contact your local Department of Health Services for information on the disposal of this product or arrange for disposal by a licensed disposal company.

TRANSPORTATION INFORMATION:

UN Number: N/A
Proper Shipping Name: N/A
IATA Class: N/A
IATA Packing Group: N/A

REGULATORY INFORMATION: X

Risk Phrases:

R21/22:	Harmful in contact with skin and if swallowed.
R36/37/38:	Irritating to eyes, respiratory system and skin.

Safety Phrases:

S7:	Keep container tightly closed.
S36/37:	Wear suitable protective clothing and gloves.

OTHER INFORMATION:

Avoid contact with material. Avoid prolonged or repeated exposure. Wash thoroughly after handling. Material should only be handled by qualified, experienced professionals. The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide for experienced personnel. **CALBIOCHEM-NOVABIOCHEM** Corporation shall not be held liable for any damage resulting from the handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.