THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 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	Dr. Joan Knoll
DEPARTMENT	Pathology
ADDRESS	Room 5013 Dental Sciences Building
PHONE NUMBER	(519) 661 2111 extension 86407
EMERGENCY PHONE NUMBER(S)	(519) 601 3399
EMAIL	joan.knoll@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) Dental Sciences Room(s)5002, 5002A, 5003

*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:_Canada Foundation of Innovation; Ontario Research Fund; OGI, CIPI, Internal

GRANT TITLE(S): Translational Cytogenomic Infrastructure for Detection and Characterization of Chromosomal Abnormalities

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

Name	UWO E-mail Address	Date of Biosafety Training
Heather Tarnowski	htarnow@uwo.ca	July 17 2008
Wahab Khan	wkhan43@uwo.ca	April 19 2011

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.

RP11 Bacterial Artificial Chromosomes and Plasmids in *Escherichia coli*

Escherichia coli containing bacterial artificial chromosomes or plasmids with human genomic/gene DNA inserts will be used in one of two ways: 1) Cultured and used immediately for extraction of bacterial artificial chromosome DNA; 2) Cultured for storage at -80°C (for future culture and DNA extraction). DNA extracted from *E.coli* will be used for fluorescent *in situ* hybridization. Waste created from *E.coli* culture will be given to RPR Environmental for incineration.

Human Lymphocyte, Fibroblast, Lymphoblastoid and Tumor Cell Lines

Cell lines will be cultured for use in fluorescent *in situ* hybridization, DNA extraction, and RNA extraction. Cells will also be cultured and stored in liquid nitrogen for future use.
Waste created from culture of these cells will be autoclaved with a Verify test, and be disposed of in the garbage.

Primary human tissue

Cells will be cultured or handled for in vitro assays and/or nucleic acids extracted from primary human tissues such as whole blood or skin. Cells will also be stored in liquid nitrogen for future use. Waste created from handling of these cells will be autoclaved with a Verify test and disposed of in the garbage.

Please include a one page research summary or teaching protocol.

Description of Research Activities:

The research in our laboratory is translational in nature with a goal of moving genetic research findings into the clinical laboratory setting. The laboratory's research activities include:

1. Phenotype/genotype correlations in inherited diseases with chromosome abnormalities; examining chromosome structure at the submicroscopic level (using fluorescent *in situ* hybridization and other molecular biology and chromosome biology methods)
2. Identifying genome copy number changes and their relationship to inherited diseases such as leukemia and acquired diseases such as leukemia and breast cancer (using molecular techniques)
3. Developing novel cytogenomic and genomic technology to improve inherited and acquired disease diagnosis

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? **O 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 **O 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)* (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
Escherichia coli	O Yes **O No**	O Yes **O No**	O Yes **O No**	Two	The Centre for Applied Genomics, Invitrogen, (Appendix A)	**O 1** O 2 O 2+ O 3
						O 1 O 2 O 2+ O 3
						O 1 O 2 O 2+ O 3
	O No	O No	O No			O 1 O 2 O 2+ O 3

DH5 alpha

*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? **O YES** O 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	**O Yes** O No	Lymphocytes and fibroblast cells from collaborating institutions	Not applicable
Rodent	O Yes **O No**		
Non-human primate	O Yes **O No**		
Other (specify)	O Yes **O 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	**O Yes** O No	Lymphoblastoid cells, fibroblast cell lines, and tumor cell lines such as BT-474, MCF-7, SKBR3, Hs-578T, T47D, and MDA-MB-231,	Level 1	BT-474; MCF-7 UWO; SKBR3; Hs-578T, T47D, MDA-MB-231 Collaborators at UWO App B; Lymphoblasts – J.Knoll UWO, ATCC, Coriell
Rodent	**O Yes** O No	Lymphoblast cell lines, fibroblast cell lines	Level 1	ATCC, Coriell
Non-human primate	O Yes **O No**			
Other (specify)	O Yes **O 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 O 1 **O 2** O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? **O YES** O 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	Collaborating genetic institutions	O Yes **O Unknown**	N/A	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)	Collaborating genetic institutions	O Yes **O Unknown**	N/A	O 1 **O 2** O 2+ O 3
Human Organs or Tissues (preserved)	Collaborating genetic institutions	Not Applicable	N/A	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? **O YES** O 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
DH5 α *Escherichia coli* (Appendix A)	PCR 2.1 TOPO PCR Blunt	Invitrogen Invitrogen (Appendix C)	*N/A*	Plasmid increases in size

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

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
O 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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES ⊗ NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES **O 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 **O 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: _____ O NO

6.5 Will the agent(s) be shed by the animal: O YES O 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 **O No** If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

7.3 If yes, 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

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

_____

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES **O 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 **O 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 **O 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_____ **O 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 _____

6/2011

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 **O 2** O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?
O YES, date of most recent biosafety inspection: March 9 2010 (MSB 365A)
O NO, please certify DSB 5002A
O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): BIO-UWO-0215

14.0 Procedures to be followed

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

If needed, we could follow the regulations for a level 2+ biohazard containment laboratory.

14.2 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:See Section 3.5 in UWO Biosafety Manual
Worker: 1) Wash exposed site 2) Inform supervisor 3) Seek medical attention 4) Provide information for incident report.
Supervisor: 1) Complete/sign incident report 2) Ensure incident is reported to human resources within 24 hours

14.3 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.wph.uwo.ca/

SIGNATURE _____ Date: May 25, 2011.

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: _____
Date: _____

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

Special Conditions of Approval:

Appendix A

RP11 - BAC Infosheet from Centre for Applied Genomics

Sherilyn Bell
TCAG Genome Resource Facility
The Hospital for Sick Children
MaRS Centre - East Tower
101 College Street, Rm 14-601H
Toronto, ON, M5G 1L7
Phone: (416) 813-8614
FAX: (416) 813-8319
e-mail: sbell@sickkids.ca

9 Aug 2010

Heather Tarnowski
Department of Biochemistry
Schulich School of Medicine and Dentistry
The University of Western Ontario
Room 0037 Dental Sciences Building
1151 Richmond Street,
London, ON
N6A 5C1

Dear Heather:

Please find enclosed the 6 human genomic clones that you requested.

RP11 human BACs:
179D12
427M4
754J8
419K10
368G9
770G7

They are sent as LB stabs. We recommend that you streak them on LB + chloramphenicol agar plates, end-sequence-verify and make glycerol stocks of each clone.

If you have any questions please do not hesitate to contact me.

Sincerely,

Sherilyn Bell

Appendix A - DH5α E.coli MSDS Sheet.

E. coli

invitrogen

Material Safety Data Sheet

Revision Date: 19-Mar-2010

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

Product code 18265017
Product name Subcloning Efficiency ™ DH5alpha™ Competent 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
The product contains no substances which at their given concentration, are considered to be hazardous to health. 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

3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
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	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

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

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:
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

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

Appendix B- Breast Cancer (Tumor) Cell Lines in Dr. J. Knoll's Lab March 2011

ATCC

Product Information Sheet for ATCC HTB-20™

Cell Line Designation: BT-474
ATCC® Catalog No. HTB-20™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: mammary gland; breast; ductal carcinoma
Age: 60 years
Gender: female
Ethnicity: Caucasian
Morphology: epithelial
Growth Properties: adherent, patchy (patches are compact multilayered colonies that rarely become confluent).
DNA profile (STR analysis):
Amelogenin: X
CSF1PO: 10,11
D13S317: 11
D16S539: 9,11
D5S818: 11,13
D7S820: 9,12
TH01: 7
TPOX: 8
vWA: 15,16
VirusSuscept: mouse mammary tumor virus (RIII-MuMTV)
Tumorigenic: yes, in nude mice; forms nodules in Amsterdam/IMR rats with regression in 10 days
Isoenzymes: G6PD, B; PGM3, 1; PGM1, 1; ES-D, 1; Me-2, 0; AK-1, 1; GLO-1, 1; Phenotype Frequency Product: 0.0426
Depositor: E.Y. Lasfargues
The BT-474 line was isolated by E. Lasfargues and W.G. Coutinho from a solid, invasive ductal carcinoma of the breast.
Karyology: The cell line is aneuploid human female (XO usually), with most chromosome counts in the hypertetraploid range.
Several chromosomes (N11, N13, and N22) are absent, and others are clearly under-represented (N9, N14, and N15) with respect to the other normal chromosomes. Chromosome N7 tends towards over-representation in several karyotypes.
Some of the missing normal chromosomes are represented by their involvement in the nine stable marker chromosomes: der(14)t(14;?)(q32;?), unknown, iso(13q), der(6)t(6;7)(q21;q21), der(11)t(11;?)(14;?), del(11)(p11), unknown, unknown, der(2)t(2;?)(p21;?). Several of the latter were reported by E. Lasfargues, et al.
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-20D™ (10µg)

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. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

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. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium. and spin at approximately 125 x g for 5 to7 minutes.
4. Resuspend 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.
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: HTB-20 recovers slowly from cryopreservation. It may take two to four weeks for the cells to reach 70-80% confluence in a T-75 flask after thaw.

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 1:3.
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 Hybri-Care Medium, Catalog No. 46-X. Hybri-Care Medium is supplied as a powder and should be reconstituted in 1 L cell-culture-grade water.
To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concent
10%
- 1.5 g/L sodium bicarbonate for use v
CO_2

ATCC tested fetal bovine serum is available
Catalog No. 30-2020.

Cryoprotectant Medium

Complete culture medium described above su
with 5% (v/v) DMSO. Cell culture tested DMSO
as ATCC Catalog No. 4-X.

Additional Information

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

Cell line info

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

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

ATCC

Product Information Sheet for ATCC HTB-20™

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Or contact your lo

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

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- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

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.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

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 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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www.atcc.org

800-638-6597 or 703-365-2700
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Or contact your local distributor.

ATCC® Product Information Sheet for ATCC® HTB-22™

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

Cell Line Designation: SK-BR-3
ATCC® Catalog No. HTB-30™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Medium Renewal
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- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: breast ;adenocarcinoma; derived from metastatic site:; malignant pleural effusion
Age: 43 years
Gender: female
Ethnicity: Caucasian
Morphology: epithelial
Growth properties: adherent
AntigenExp: Blood Type A; Rh+; HLA A11, Bw22(+/-), B40, B18
Tumorigenic: yes, in nude mice; forms poorly differentiated adenocarcinoma
DNA profile (STR analysis)

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

Depositors: G. Trempe; L.J. Old
Comments: This cell line was derived by G. Trempe and L.J. Old in 1970 from a pleural effusion. Ultrastructural features include microvilli and desmosomes, glycogen granules, large lysosomes, bundles of cytoplasmic fibrils. No virus particles
The SK-BR-3 cell line overexpresses the HER2/c-erb-2 gene product.
Karyotype: This is a hypertriploid human cell line with the modal chromosome number of 84, occurring in 34% of cells. Cells having 80 chromosomes also occurred at a high rate (28%); the higher ploidy cells occurred at 7.3%.
This cell line has a very complex chromosome composition. Thirty-five to 40% of chromosomes in a cell complement with a modal chromosome number of 84 consisted of structurally altered marker chromosomes. Several markers are longer than chromosome N1.
The origins of most of these markers, however, are not clear. Some markers may have at least three individual chromosome segments.
The markers [i.e., ?der(1)t(1;21) (p13;q21) [or ?t(1q21q)], ?del(2) (q13), and t(7pter--cen--?), present in some cells only] were the only ones in which portions of chromosome segments could be identified. Most cells had about three normal X chromosomes and five or more N7. The structurally normal N1, N14 and N17 were generally absent.
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-30D (10 micrograms)

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

Use 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.
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) 639-3620; FAX (212) 753-5764

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. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium. and spin at approximately 125 xg for 5 to7 minutes.
4. Resuspend 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 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 1:3
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 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%

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 and ATCC Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete culture 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)

Fogh, J., **ed., Human tumor cells in vitro.** New York: Plenum Press; 1975:pp. 115-159

Cell Line Designation: HS 578T
ATCC® Catalog No. HTB-126™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: mammary gland; breast
Age: 74 years
Gender: female
Ethnicity: Caucasian
Morphology: epithelial
Growth properties: adherent
DNA profile (STR analysis):
Amelogenin: X
CSF1PO: 13
D13S317: 11
D16S539: 9,12
D5S818: 11
D7S820: 10
TH01: 9,9.3
TPOX: 8
vWA: 17

Tumorigenic: no; The cells were not tumorigenic in immunosuppressed mice, but did form colonies in semisolid medium.
Depositors: A.J. Hackett
Comments: The Hs 578T cell strain was derived from a carcinoma of the breast. It was originated by A.J. Hackett, et al. along with the Hs 578Bst (see ATCC HTB-125), which is a normal fibroblast-like line from the same patient.
The Hs 578T line had a mixed polygonal morphology initially, but a stellate cell type was selected for during passage and by cloning.
Aggregates of casein protein granules, desmosomes, tight junctions, lipid droplets and vesicularized smooth endoplasmic reticulum were observed by electron microscopy.
As with Hs 578Bst, no estrogen receptors or endogenous viruses were detected.
Karyotype: Number of cells examined = 50; Modal Chromosome Number = 59 with a range of 50 to 77; Polyploidy Rate = 33.8%
Composite karyotype: 50-77 <3n> X, -1, del(1)(q12), -2, del(2)(?q36), der(3)t(3;15)(q10;p10),-4, -5,der(5)t(5;8)(p10;q10),-6, i(6)(p10), +8, -9, -10, -11, del(11)(p12), -12, -13, -14, -15, -15, -16, -17, -17, -17, i(17)(q10), -18, -19, der(19)(19pter<-q13::5q13<-qter), +22, +3 mar[cp12]

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-126D (10µg)
Total RNA from this line is available as ATCC HTB-126R (100 µg).

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

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

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).

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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 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.

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 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 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: 1:3 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 Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002.
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%

This medium is formulated for use with a 5% CO_2 in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO_2 in air atmosphere is then recommended).
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**)

Hackett AJ et al. **Two syngeneic cell lines from human breast tissue: the aneuploid mammary epithelial (Hs 578T) and the diploid myoepithelial (Hs 578Bst) cell lines.** J. Natl. Cancer Inst. 58: 1795-1806, 1977 PubMed: 77190209

Smith HS. **In vitro properties of epithelial cell lines established from human carcinomas and nonmalignant tissue.** J. Natl. Cancer Inst. 62: 225-230, 1979 PubMed: 79112564

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

800-638-6597 (U.S., Canada, and Puerto Rico)
703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org

Cell Line Designation: T-47D
ATCC® Catalog No. HTB-133™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: mammary gland; breast ductal carcinoma; derived from metastatic site: pleural effusion
Age: 54 years
Gender: female
DNA profile (STR analysis):
Amelogenin: X
CSF1PO: 11,13
D13S317: 12
D16S539: 10
D5S818: 12
D7S820: 11
TH01: 6
TPOX: 11
vWA: 14
Morphology: epithelial
Growth properties: adherent
DoublingTime: 32 hrs
Receptors: calcitonin; androgen receptor, positive; progesterone receptor, positive; glucocorticoid; prolactin; estrogen receptor, positive.
Isoenzymes: AK-1, 1; ES-D, 2; G6PD, B; GLO-I, 1-2; PGM1, 1; PGM3, 1
Tumorigenic: forms colonies in soft agar
Depositors: I. Keydar
Comments: The T-47 line was isolated by I. Keydar from a pleural effusion obtained from a 54 year old female patient with an infiltrating ductal carcinoma of the breast.
This differentiated epithelial substrain (T-47D) was found to contain cytoplasmic junctions and receptors to 17 beta estradiol, other steroids and calcitonin.It will form colonies in soft agar.
The cells express the WNT7B oncogene [PubMed: 8168088].
Cytogenetic analysis: This is a hypotriploid human cell line. The modal chromosome number is 65 occurring at 50% and polyploidy at 0.8%. 18 marker chromosomes are common to most cells, of which 7 are paired and 11 are single-copied. The t(8q14q), t(9q17q), t(10q17p) are among 7 paired markers common to most cells. N7, N9, and N10 are absent and N11 is generally present in 4 copies. DM's occurred, but infrequently. Q-band examination did not show the presence of a Y chromosome.

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-133D (10µg).
Total RNA from this line is available as ATCC HTB-133R (100µg)

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

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

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).

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ATCC®

Product Information Sheet for HTB-133™

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. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium, and spin at approximately 125 xg for 5 to7 minutes.

4. Resuspend 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 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- 0.53 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.
Subcultivation Ratio: 1: 3 to 1: 5

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 RPMI-1640 Medium, Catalog No. 30-2001.
To make the complete growth medium, add the following components to the base medium:
- 0.2 Units/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.
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

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)

Keydar I et al. **Establishment and characterization of a cell line of human breast carcinoma origin**. Eur. J. Cancer 15: 659-670, 1979 PubMed: 80068971

Judge SM and Chatterton RT Jr. **Progesterone-specific stimulation of triglyceride biosynthesis in a breast cancer cell**

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Cell Line Designation: MDA-MB-231
ATCC® Catalog No. HTB-26™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: mammary gland; breast adenocarcinoma; derived from metastatic site: pleural effusion
Age: 51 years
Gender: female
Ethnicity: Caucasian
Tumorigenic: yes, in nude mice also in ALS treated BALB/c mice; forms poorly differentiated adenocarcinoma (grade III)
AntigenExp: Blood Type O; Rh-
DNA profile (STR analysis):
Amelogenin: X
CSF1PO: 12,13
D13S317: 13
D16S539: 12
D5S818: 12
D7S820: 8,9
TH01: 7,9.3
TPOX: 8,9
vWA: 15,18
Morphology: epithelial
Growth properties: adherent
Receptors expressed: epidermal growth factor (EGF); transforming growth factor alpha (TGF alpha)
Depositors: R. Cailleau
Comments: The cells express the WNT7B oncogene [PubMed: 8168088].
Karyology: The cell line is aneuploid female, with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al., Cancer Genet. Cytogenet. 3: 61, 1981.

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 Catalog HTB-26D™ (10µg)
Total RNA from this line is available as ATCC HTB-26R™ (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.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

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. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 xg for 5 to 7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a new culture flask.

5. Incubate the culture at 37°C in a suitable incubator. **(without CO2)**

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 free gas exchange with atmospheric air 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° in a free gas exchange with atmospheric air 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 with 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 cell suspension to new culture vessels.
Subcultivation Ratio: 1:2 to 1:4.

6. Place culture vessels in incubators 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

2 to 3 times weekly.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008.
To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

Note: The L-15 medium formulation was devised for use in a free gas exchange with atmospheric air. A CO_2 and air mixture is detrimental to cells when using this medium for cultivation
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

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 are available in the catalog at www.atcc.org)

Brinkley BR et al. **Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro**. Cancer Res. 40: 3118-3129, 1980 PubMed: 81042058

Cruciger Q et al. **Morphological, biochemical and chromosomal characterization of breast tumor lines from pleural effusions**. In Vitro 12: 331, 1976

Siciliano MJ et al. **Mutually exclusive genetic signatures of human breast tumor cell lines with a**

;r formation For Lymphoblast
Cell Lines In Knoll Lab (Appendix B)

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Catalog Tools:

Catalog ID: **GM05372** Product (Source): CELL CULTURE ADD TO CART

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Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Inherited Disorders
Class Mutations of the Hemoglobin Loci
Sample Description HUMAN ERYTHROLEUKEMIC CELL LINES CHRONIC MYELOGENOUS LEUKEMIA
Biopsy Source Peripheral vein
Cell Type B-Lymphocyte
Tissue Type Blood
Transformant Epstein-Barr Virus
Species Homo sapiens
Common Name Human
Age At Sampling 53 YR
Sex Female
Race Caucasian
Family 222
Family Member 1
Relation to Proband proband
Clinically Affected Yes
Confirmation Clinical summary/Case history
Remarks Line K562; established from a patient with chronic myelogenous leukemia by Lozzio and Lozzio; produces embryonic hemoglobin in response to hemin; modal number of chromosomes = 60; range is 55-62; structural aberrations for chromosomes #1, #3, #9, #13, and #14 were observed

Catalog ID GM05372
Product Cell Culture
Pricing Commercial Pricing: $85.00
Academic and not-for-profit pricing: $85.00
How to Order Online Ordering
Assurance Form (Must have current form on file)
Statement of Research Intent Form (Information will be entered electronically when order is placed. DO NOT fax form to Coriell Customer Service)

Characterizations

Sample Description HUMAN ERYTHROLEUKEMIC CELL LINES CHRONIC MYELOGENOUS LEUKEMIA

IDENTIFICATION OF SPECIES OF ORIGIN Species of Origin Confirmed by Nucleoside Phosphorylase, Glucose-6-Phosphate Dehydrogenase, and Lactate Dehydrogenase Isoenzyme Electrophoresis and by Chromosome Analysis

Phenotypic Data

Remark Line K562; established from a patient with chronic myelogenous leukemia by Lozzio and Lozzio; produces embryonic hemoglobin in response to hemin; modal number of chromosomes = 60; range is 55-62; structural aberrations for chromosomes #1, #3, #9, #13, and #14 were observed

Publications

Gervais FG, Thornberry NA, Ruffolo SC, Nicholson DW, Roy S, Caspases cleave focal adhesion kinase during apoptosis to generate a FRNK-like polypeptide. J Biol Chem273:17102-8 1998
PubMed ID: 9642276

ten Hoeve J, Morris C, Poustka A, Groffen J, Heisterkamp N, Isolation of NotI sites from chromosome 22q11. Genomics18:588-97 1993
PubMed ID: 7905853

Mononen I, Heisterkamp N, Kaartinen V, Mononen T, Williams JC, Groffen J, Aspartylglycosaminuria in a non-Finnish patient caused by a donor splice mutation in the glycoasparaginase gene. J Biol Chem267:3196-9 1992
PubMed ID: 1737774

Mononen I, Heisterkamp N, Kaartinen V, Williams JC, Yates JR 3d, Griffin PR, Hood LE, Groffen J, Aspartylglycosaminuria in the Finnish population: identification of two point mutations in the heavy chain of glycoasparaginase. Proc Natl Acad Sci U S A88:2941-5 1991
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Suedhoff T, Birckbichler PJ, Lee KN, Conway E, Patterson MK Jr, Differential expression of transglutaminase in human erythroleukemia cells in response to retinoic acid. Cancer Res50:7830-4 1990
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Hess J, Perez-Stable C, Deisseroth A, Shen CK, Characterization of an unique RNA initiated immediately upstream from human alpha 1 globin gene in vivo and in vitro: polymerase II- dependence, tissue specificity, and subcellular location. Nucleic Acids Res13:6059-74 1985
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Shipp MA, Schwartz BD, Kannapell CC, Griffith RC, Scott MG, Ahmed P, Davie JM, Nahm MH, A unique DR-related B cell differentiation antigen. J Immunol131:2458-67 1983
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Machado EA, Mitchell JR, Lozzio BB, Lozzio CB, Gerard DA, Absence of morphological, chromosomal and antigenic changes in the K- 562 cell line growing as localized or disseminated tumours in nude mice. Br J Cancer46:383-91 1982
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Lozzio BB, Lozzio CB, Bamberger EG, Feliu AS, A multipotential leukemia cell line (K-562) of human origin. Proc Soc Exp Biol Med166:546-50 1981
PubMed ID: 7194480

Carrel S, Gross N, Salvi S, Rabbit antiserum against Daudi-cell membrane fractions detects cell surface antigens present on subpopulations of human lymphocytes. J Immunol120:1587-93 1978
PubMed ID: 307919

Lozzio CB, Lozzio BB, Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood45:321-34 1975
PubMed ID: 163658

External Links

dbSNP dbSNP ID: 15466
OMIM 606252 LEUKEMIA, CHRONIC MYELOID; CML

Images

Data are not available

Culture Protocols

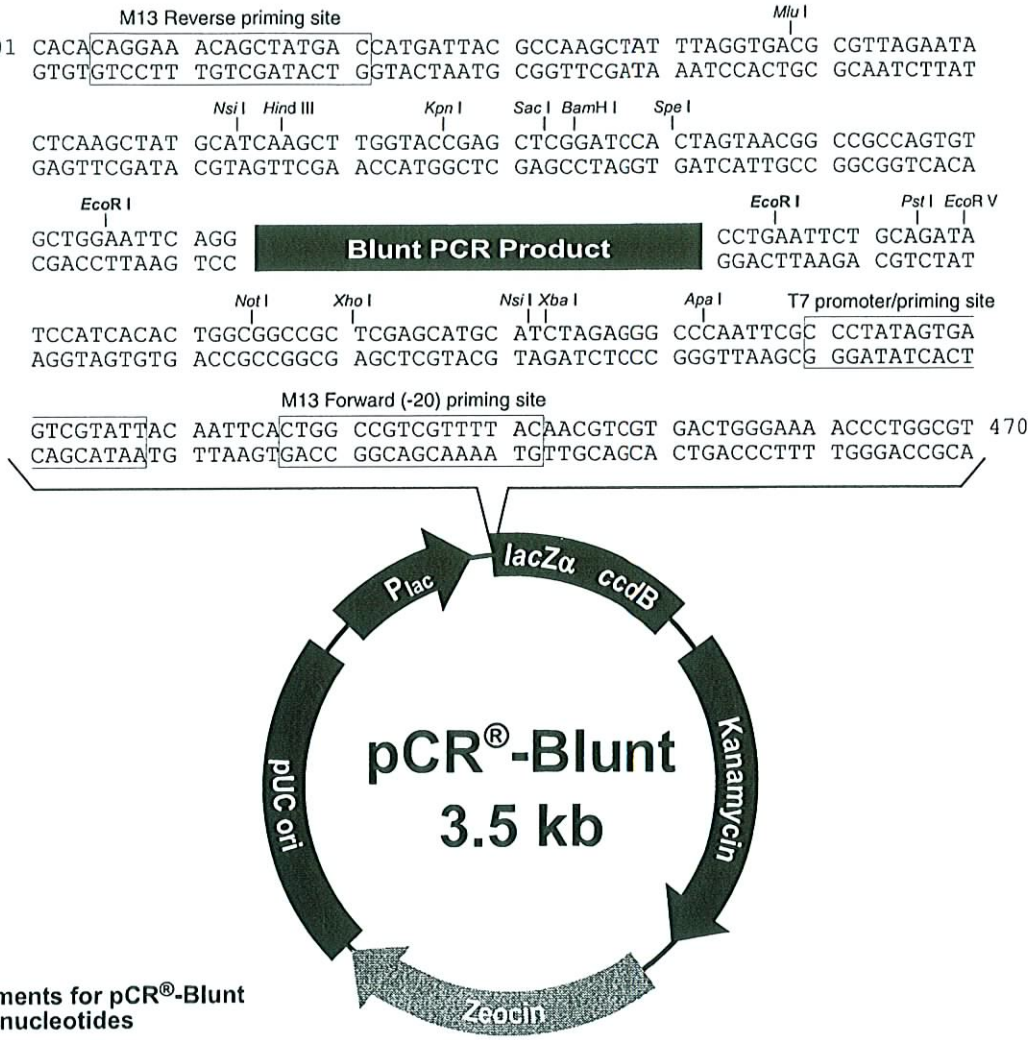
Split Ratio 1:3
Temperature 37 C
Percent CO2 5%
Medium Roswell Park Memorial Institute Medium 1640 with 2mM L-glutamine
Serum 15% fetal bovine serum Not Inactivated
Substrate None specified
Subcultivation Method dilution - add fresh medium

Plasmid(s)

Map and Features of pCR®-Blunt

Map of pCR®-Blunt

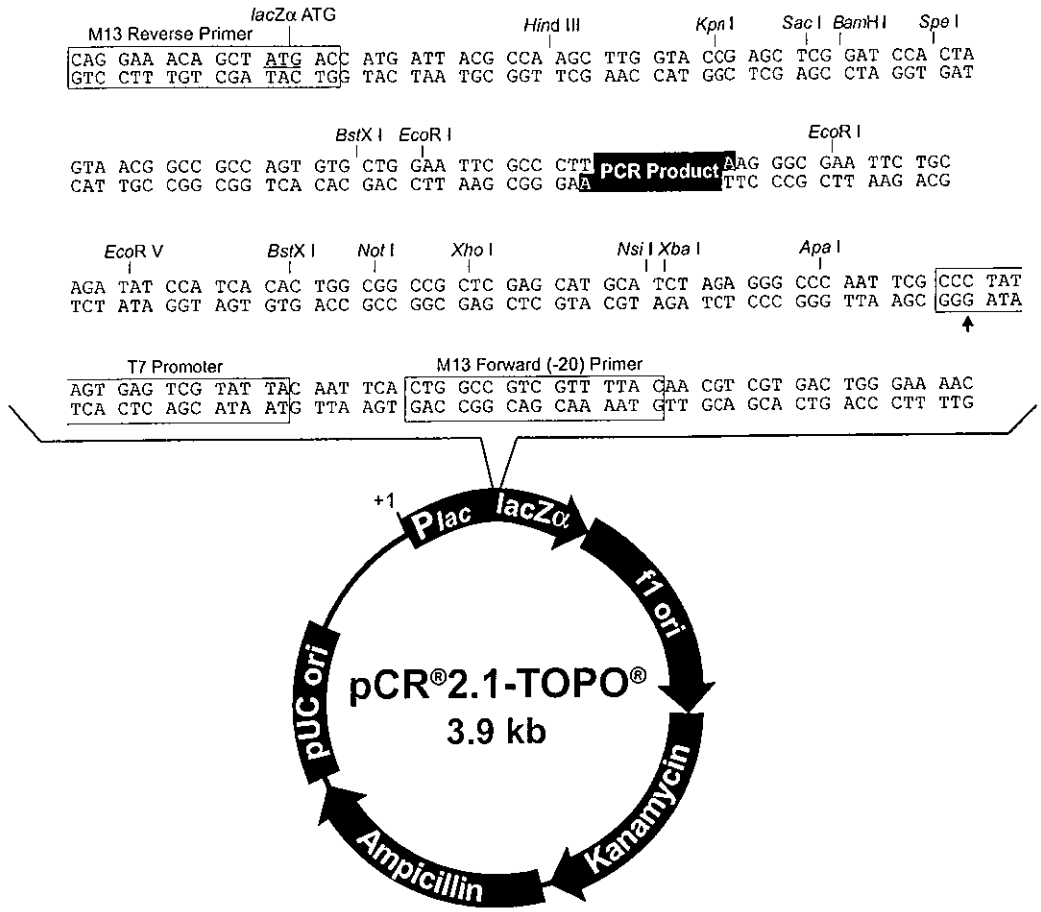
The figure below summarizes the features of the pCR®-Blunt vector. Restriction sites that are only found in the polylinker are shown. **The complete sequence is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 19).**



Comments for pCR®-Blunt
3512 nucleotides

Lac promoter/operator region: bases 95-216
M13 Reverse priming site: bases 205-221
*Lac*Z-alpha ORF: bases 217-570
T7 promoter priming site: bases 400-419
M13 Forward (-20) priming site: bases 427-442
Fusion joint: bases 571-579
*ccd*B lethal gene ORF: bases 580-882
Kanamycin resistance ORF: bases 1231-2025
Zeocin resistance ORF: bases 2231-2605
pUC origin: bases 2673-3386

continued on next page



Comments for pCR®2.1-TOPO®
3931 nucleotides

LacZα fragment: bases 1-547
M13 reverse priming site: bases 205-221
Multiple cloning site: bases 234-357
T7 promoter/priming site: bases 364-383
M13 Forward (-20) priming site: bases 391-406
f1 origin: bases 548-985
Kanamycin resistance ORF: bases 1319-2113
Ampicillin resistance ORF: bases 2131-2991
pUC origin: bases 3136-3809

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