Modification Form for Permit BIO-UWO-0017

Permit Holder: Dale Laird

PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOLOGICAL AGENTS.
PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOLOGICAL AGENTS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF.

Approved Personnel
(Please stroke out any personnel to be removed)

Tao Huang
~~Wesley Lai~~ → REMOVE
Mark Ableser
Shreya Podder
Amy Berger
Kevin Barr
Jamie Simek
Xiang-Qun Gong
Silvia Penuela
Cindy Shao
Michael Stewart
John Kelly

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biological Agent(s) to be removed	**Write additional Biological Agent(s) for approval below. Give the full name**
Approved Microorganisms	E. coli DH5 alpha, JM109, adenovirus, retrovirus	
Approved Primary and Established Cells	Human (primary), skin biopsies, Rodent (primary), genetically modified mice, fibroblasts, Human (established), HELA, 293T, 293 Hek, TE 354.T, Hs 456.Sk, TE 353.Sk, CCD-1074Sk, SC.ZR75, Hs578T,	C81G, C81-61, A2058 A375, HEMa-LP, HEMn-LP HEMn-DP
Approved Use of Human Source Material	Human blood (whole) or other body fluid: ODDD Patients and relatives. Human Organs orTissues (unpreserved): ODDD Patients and relatives. Human Organs orTissues (preserved): ODDD Patients and relatives.	
Approved Genetic Modifications (Plasmids/Vectors)	[plasmids]T-East, pcDNA3, pEGFP, pc DNA-mRFP, pTagRFP vector, CMV-R-GECO1, pRc/CMC, Tet-ON/off Cherry, pEBTet GFP. [vectors]: AP-2, pRNA-H1.1	

Approved Use of Animals	mice	
Approved Biological Toxin(s)	cholera	
Approved Gene Therapy		
Approved Plants and Insects		

As the Principal Investigator, I have ensured 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/newposition.htm

Signature of Permit Holder: _____

Current Classification: 2 **Containment Level for Added Biohazards:** 2

Date of Last Biohazardous Agents Registry Form: May 2, 2012

Date of Last Modification (if applicable): _____

BioSafety Officer(s)*:

***For work being performed at Institutions affiliated with Western University, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Western University Biosafety Officer.**

Chair, Biohazards Subcommittee: _____ **Date:** _____

Dale Laird - Re: Grant Approvals for Work Involving Biohazardous Agents (Laird) - MODIFICATION

From: Jennifer Stanley <jstanle2@uwo.ca>
To: Dale Laird <Dale.Laird@schulich.uwo.ca>
Date: 27/07/2012 4:40 PM
Subject: Re: Grant Approvals for Work Involving Biohazardous Agents (Laird) - MODIFICATION
CC: Cindy Shao <Cindy.Shao@schulich.uwo.ca>, Silvia Penuela <Silvia.Penuela@...
Attachments: Laird_July_2012.pdf; Modification_Information_January_2012.pdf

Hi there

Please complete the attached modification form for the new cells.

Regards
Jennifer

On 7/27/2012 1:15 PM, Dale Laird wrote:

> Dear Jennifer,
>
> Sorry for the delay in getting back to you. In addition to the 5 year CIHR grant you note below we also had a 3 year Canadian Cancer Society Research Innovation (CCSRI) grant called "**The novel channel protein Pannexin 1 as a viable target for melanoma treatment**" approved to study melanocytes and melanomas, so I will cover off a few additions to our Biohazard registry form for both grants. Please add both grant titles to my registry.
>
> For the CIHR grant, we are covered for all cells and reagents used in this study with the exception that you should add spontaneously immortalized dermal fibroblasts from humans and primary human keratinocytes that are immortalized by treating with a Rho Kinase inhibitor.
>
> For the CCSRI grant please add the following cell lines.
>
> From ATCC, we will be using the human melanoma cell lines [redacted], A2058 and A375. C8161 and C81-61 cell lines are isogenic lines. The C8161 is highly tumourigenic, poorly differentiated (unpigmented) and invasive. The C81-61 is not tumourigenic, is well differentiated (pigmented) and poorly invasive. Both the A2058 and A375 are tumorigenic.
>
> From Cascade we will use the human primary epidermal melanocyte lines HEMa-LP, HEMn-LP and HEMn-DP. These lines are not transformed.

In keeping with past practices we treat all our cells lines under containment level 2 even though that maybe overkill for some lines.

Please let me know if you need any further information to add these changes.

Best regards,

Dale

Dale W. Laird, Ph.D.
Professor
Canada Research Chair in Gap Junctions and Disease
Department of Anatomy and Cell Biology
University of Western Ontario
Dental Science Building, Rm 00077
London, Ontario, Canada, N6A-5C1
Tel: (519) 661-2111 x86827
Fax: (519) 850-2562
Dale.Laird@schulich.uwo.ca
www.uwo.ca/anatomy/laird/index.htm

>>> Jennifer Stanley <jstanle2@uwo.ca> 19/07/2012 5:01 PM >>>
Re: Grant Approvals for Work Involving Biohazardous Agents

Hello there:

Congratulations (see below).

One or more of your grants for work involving biohazardous agents was recently approved by the Western University Biosafety Officer.

Please note that the biohazards of this research must be the same as the biohazards in your current, valid Biological Agents Registry Form.

If there are new biohazards in this new work, please let me know and we will modify your current form on file.

For more information, please see:
http://www.uwo.ca/humanresources/facultystaff/h_and_s/biosafety/biosafety_idx.htm

Regards,
Jennifer

-------- Original Message --------
Subject: Biohazard Certification Review Required: Work List Item
Date: Tue, 17 Jul 2012 08:10:15 -0400 (EDT)
From: PeopleSoft Grants Management <psoft@uwo.ca>
To:jstanle2@uwo.ca

The following Award has been accepted:

Sponsor : CANADIAN INSTITUTES HEALTH RESEARCH
Program : 00100
PI : Laird,Dale W
Award : R2791A16
Title : Role of Cx26 and Cx30 in skin aging

Funding from the agency has been approved for the above noted Award. The PI has indicated that the research will require Biohazard certification. Please confirm the certification information provided by the PI. This information will need to be completed before the Award is activated. Please follow the link to view the work list item and confirm the information supplied by the PI. To clear this item from you work list, check the box next to Certification OK

You may access the information by clicking here.

Note: Do not respond to this email. For further information please contact Research Services.

damage. Finally, we will use a chick model system to determine how pannexin 1 causes tumor cells to migrate into the liver and lungs. Collectively, these studies will define how these unique channels promote cancers to form and become aggressive.

13. Public summary - impact and relevance statement

Detail in 2-3 sentences (maximum of 1000 characters) how your studies will contribute to the reduction of cancer incidence rates for Canadians and/or cancer mortality rates for Canadians and/or enhanced quality of life for Canadians living with and beyond cancer.

Malignant melanoma is becoming more prevalent each year and although it accounts for only 4% of all skin cancers, it is responsible for 79% of skin cancer-related deaths. Consequently, melanomas that exit the skin and enter vital organs are one of the most deadly cancers. Clinically, this problem is exasperated by the shortage of clinical dermatologists in Canada leading to patients frequently being diagnosed with metastatic disease upon initial assessment of suspicious lesions. Our studies are designed to evaluate whether a channel, found in high levels on the surface of melanoma cells, may in fact be a suitable target for treatment. What makes this potential target an exciting idea is the fact that its channel activity can be blocked by small molecules making it a very suitable target for drug development. The current proposal will clearly establish if the protein, pannexin 1, that is used to build these novel channels would be a viable target for therapeutic drug development.

Abstract

14. Scientific abstract

Provide a detailed summary of your research project (maximum of 4200 characters), stating the problem to be investigated, the objectives of the investigation, the methodology to be used, as well as the significance of the research to cancer.

The novel channel-forming protein Pannexin 1 as a viable target for melanoma treatment

Pannexins (Panx) are a novel family of three channel forming glycoproteins. Panx1 is ubiquitously expressed in mammalian organs and forms a functional channel for ATP release. Panx1-mediated ATP release upon activation by caspase cleavage was shown to act as a "find-me" signal for thymocyte clearance by macrophages in a recent Nature paper that we co-authored.

Malignant melanoma is one of the most deadly cancers accounting for ~79% of all skin cancer-related deaths. Analysis of a limited sample archive reported in the Human Protein Atlas revealed that Panx1 is up-regulated in ~70% of all human melanoma tumors samples. High levels of Panx1 were also found in lung, colorectal and non-melanoma skin cancer samples suggesting that Panx1 may be important in tumor onset and/or progression. Our preliminary data indicated that Panx1 is expressed at low levels in skin melanocytes and is highly upregulated in malignant melanoma cells. Panx1 levels were further found to be positively correlated with the aggressiveness of B16 isogenic melanoma cell lines. Upon Panx1-knockdown, melanoma cells resemble normal melanocytes in cell morphology and melanin production, exhibiting reduced migration and growth. *In vivo,* Panx1-depleted cells formed smaller melanoma tumors in the chick chorioallantoic membrane and had significantly reduced metastasis to the liver of the avian

embryos. Therefore, we propose that Panx1 is significantly up-regulated as melanocytes transform into melanomas and, moreover, a targeted knockdown of Panx1 can reduce the tumorigenic properties of melanomas. *We hypothesize that Panx1 acts as a proto-oncogene in melanocyte transformation and is a viable therapeutic target in the prevention of melanoma disease progression.*

Our specific aims are:

1) Determine at what stage of melanoma progression Panx1 is up-regulated in human melanomas and correlate these findings with clinical history and patient prognosis.
2) Examine if Panx1 has proto-oncogenic properties by establishing if it can transform melanocytes when over-expressed.
3) Elucidate if Panx1 up-regulation acts to recruit macrophages to the melanoma tumor sites to enhance growth and angiogenesis through ATP release *in vivo*.

Aim 1: We will use immunohistochemistry to assess Panx1 expression in normal human skin sections and biopsies from melanoma lesions at stages 0 to 4 through accessing the tumor bank at the London Health Science Centre and by analysing commercial tumor microarrays. We will compare Panx1 expression and tissue distribution in human samples with available clinical data to determine potential correlations with melanoma stage, prognosis and metastasis.
Aim 2: We will examine Panx1 expression in human and mouse melanocytes and melanoma cells. Through ectopic expression and shRNA knockdown we will engineer stable melanocyte and melanoma cell lines with high and low levels of Panx1 to evaluate phenotypic cell changes and the expression of markers of malignant melanoma. Susceptibility of Panx1-overexpressing melanocytes to transformation upon UV irradiation will be assessed and compared to controls. Finally, Panx1-null (in-house) and control mice will be subjected to UVB radiation to determine if they are less susceptible to the formation of melanomas.
Aim 3: We will correlate Panx1 expression levels and ATP release with melanoma tumor growth in xenograft chick chorioallantoic membrane assays and quantify metastasis using qPCR of the excised avian embryonic organs. Since Panx1 can form channels that release 'find-me' signals to macrophages, we will test if high levels of Panx1 can result in the recruitment of tumor-associated macrophages that promote tumor growth and angiogenesis.
Together, these studies will provide clear insights into whether Panx1 is a proto-oncogene that is up-regulated in melanocytes triggering their transformation into melanomas. Since tumorigenesis and metastasis can be inhibited by the knockdown of Panx1, this raises the possibility that Panx1 may be a viable target for the treatment of melanoma.

15. Keywords/Technical terms

Provide up to a maximum of ten specific keywords or descriptive technical terms/methodologies that best describe the scientific and technical aspects of your project. NOTE: Enter one keyword or technical term per line.

16. Innovation statement

CIHR GRANT

Laird, Dale | Role of Cx26 and Cx30 in skin aging | $160,854 | Summary

The human connexin family of gap junction proteins consists of 21 members. Nearly all mammalian cells express two or more members of this family which are important in establishing the direct intercellular exchange of small metabolic and signaling molecules. The skin is a particularly complex organ as keratinocytes can express upwards of 9 distinct connexins. While connexins have been linked to epidermis development and differentiation in the young it is not known how connexin expression regulates the differentiation and function of aged skin. Intriguingly, over the past decade autosomal dominant and/or recessive mutations in connexin family members have been definitively linked to a plethora of skin diseases that typically emerge during aging. Due to putative compensatory mechanisms or inhibitory cross-talk between connexin mutants and co-expressed connexins, correlating connexin gene mutations to clinical presentation of disease during aging is complex and difficult to predict. Cx26 (*GJB2*) and Cx30 (*GJB6*) gene mutations are responsible for the vast majority of connexin-linked skin diseases that are undetected in the young and only emerge during aging. The focus of the present proposal is to understand the role of connexins in the aging epidermis and to elucidate the mechanisms associated with connexin mutations and skin diseases. *Thus, we hypothesize that connexins play an essential role in epidermal differentiation and function and that connexin mutants selectively and trans-dominantly inhibit other connexins co-expressed in the epidermis to cause disease in aging skin.*

Our specific aims are to:

1) Characterize the functional, dominant and trans-dominant properties of human skin disease-linked Cx26 and Cx30 mutants in reference cell models and keratinocytes.
2) Examine the effect of connexin mutants on epidermal differentiation and function in primary keratinocytes, organotypic epidermis, mutant mice and in human models of human skin disease.

In the first aim we will use molecular, fluorescent, biochemical and electrophysiological approaches to fully characterize the trafficking, assembly, turnover and function of untagged and GFP-tagged Cx26 and Cx30 mutants that are known to cause human skin disease in aging patients. Mutant connexins will be examined in gap junctional intercellular communication–deficient N2A and HeLa cells as well as in tissue relevant rat epidermal keratinocytes. Syndromic and non-syndromic autosomal dominant Cx26/Cx30 mutations will be further assessed for their ability to promote cell death. Stable cell lines will be generated and used to mimic *in vivo* expression levels of both wild-type and mutant connexins. Dominant and trans-dominant properties of the mutants will be assessed to determine possible effects on co-expressed connexins. This aim will determine the fundamental properties of disease-linked connexin mutants and their cross-talk potential with other connexin family members.

In the second aim we examine the effect of connexin mutants on epidermal differentiation and function in primary keratinocytes, organotypic epidermis, mutant mice and in human models of human skin disease. These studies will include primary cultures of keratinocytes from three novel heterozygous mutant mouse models ($Cx26^{D66H}$, $Cx26^{S17F}$ and $Cx30^{A88V}$), two of which are already known to mimic human skin disease. The skin of mutant expressing mice will also be evaluated for the onset and severity of disease during aging. Mutant expressing immortalized keratinocytes will be assessed in organotypic cultures for cell proliferation, differentiation and cell death by 3-dimensional microscopic analysis and for the expression of markers associated with epidermal differentiation. We will also investigate calcium spread amongst primary keratinocytes from mutant and control mice to determine if the mutant-induced changes in skin phenotypes are mechanistically linked to calcium exchange. Finally, we will obtain fresh human skin biopsies from patients of different ages harboring Cx26 or Cx30 mutations and unaffected relatives and establish primary keratinocyte cell lines. These human models of connexin-linked skin disease will be assessed for possible connexin-based compensatory mechanisms and their ability to proliferative, differentiate and undergo cell death.

The C816 and C81-61 are human melanomas that were originally obtained by Dr. Lynne Postovit from Dr. Mary Hendrix's laboratory. These cells were kindly provided to us by Dr. Postovit and do not have an MSDS sheets but will be used under Biosafety level 2 containment. Dr. Postovit has included these cells under her permit (0194).

Cell Biology

ATCC® Number: CRL-11147™ Order this Item

A 2058

Price: **$431.00 (for-profit list price)**
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Designations:	A2058
Depositors:	W Stetler-Stevenson
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial
Source:	**Organ:** skin **Disease:** melanoma **Derived from metastatic site:** lymph node
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
Receptors:	nerve growth factor (NGF), expressed laminin, expressed
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 13,14 D16S539: 9,13 D5S818: 9,12 D7S820: 11 THO1: 7,9 TPOX: 8 vWA: 14,18
Age:	43 years adult
Gender:	male
Ethnicity:	Caucasian
Comments:	This cell line is highly invasive and provides a source of cellular invasion associated proteins (such as the 72000 dalton type IV collagenase. Tissue inhibitor of metalloproteinase-2 [TIMP-2], autocrine motility factor and the 67000 dalton laminin receptor.

Related Links

- NCBI Entrez Search
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement New!
- Technical Support
- Related Cell Culture Products

BioProducts

Cell, microbial and molecular genomics products for the life sciences

BioServices

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

BioStandards

Biological Reference Material and Consensus Standards for the life science community

Propagation:

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

Subculturing:

Protocol:

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

Preservation:

Subcultivation Ratio: A subcultivation ratio of 1:6 to 1:12 is recommended
Medium Renewal: Every 2 to 3 days

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

Related Products:

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

References:

22590: Fabricant RN, et al. Nerve growth factor receptors on human melanoma cells in culture. Proc. Natl. Acad. Sci. USA 74: 565-569, 1977. PubMed: 265522
23263: Sherwin SA, et al. Human melanoma cells have both nerve growth factor and nerve growth factor-specific receptors on their cell surfaces. Proc. Natl. Acad. Sci. USA 76: 1288-1292, 1979. PubMed: 375235
23269: Todaro GJ, et al. Transforming growth factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. Proc. Natl. Acad. Sci. USA 77: 5258-5262, 1980. PubMed: 6254071
23404: Stetler-Stevenson WG, et al. The activation of human type IV collagenase proenzyme. Sequence identification of the major conversion product following organomercurial activation. J. Biol. Chem. 264: 1353-1356, 1989. PubMed: 2536363
23549: Stetler-Stevenson WG, et al. Tissue inhibitor of metalloproteinase (TIMP-2). A new member of the metalloproteinase inhibitor family. J. Biol. Chem. 264: 17374-17378, 1989. PubMed: 2793861

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ATCC™

Product Information Sheet for CRL-1619

Cell Line Designation: A-375
ATCC® Catalog No. CRL-1619

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: skin; malignant melanoma
Age: 54 years
Gender: female
DNA profile (STR analysis):
Amelogenin:X
CSF1PO:11,12
D13S317:11,14
D16S539:9
D5S818:12
D7S820:9
TH01:8
TPOX:8,10
vWA: 16,17
Growth Properties: adherent
Tumorigenic: yes, in immunosuppressed mice
Depositors: D. J. Giard

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).

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 75 cm^2 tissue culture flask and dilute with the recommended complete culture medium (see the specific batch information for the recommended dilution ratio). *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).*

4. 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.

If it is desired that the cryoprotective agent be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cells with fresh growth medium at the dilution ratio recommended in the specific batch information.

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

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 supension 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

Every 2 to 3 days.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002.

To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

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.

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

Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP. **In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors.** J. Natl. Cancer Inst. 51:1417-1423, 1973. PubMed: 4357758

Gershwin ME, Ikeda RM, Kawakami TG, Owens RB. **Immunobiology of heterotransplanted human tumors in nude mice.** J. Natl. Cancer Inst. 58:1455-1463, 1977. PubMed: 857033

Muldoon RR, Levy JP, Kain SR, Kitts PA, Link CJ Jr.. **Tracking and quantitation of retroviral-mediated transfer using a completely humanized, red-shifted green fluorescent protein gene.** BioTechniques 22:162-167, 1997. PubMed: 8994663

de Vries TJ, Fourkour A, Wobbes T, Verkroost G, Ruiter DJ, van Muijen GN. **Heterogeneous expression of immunotherapy candidate proteins gp100, MART-1, and tyrosinase in human melanoma cell lines and in human melanocytic lesions.** Cancer Res. 57:3223-3229, 1997. PubMed: 9242453

Auguste P, Guillet C, Fourcin M, Olivier C, Veziers J, Pouplard-Barthelaix A, Gascan H. **Signaling of type II oncostatin M receptor.** J. Biol. Chem. 272:15760-15764, 1997. PubMed: 9188471

Shimomura T, Denda K, Kitamura A, Kawaguchi T, Kito M, Kondo J, Kagaya S, Qin L, Takata H, Miyazawa K, Kitamura N. **Hepatocyte Growth Factor Activator Inhibitor, a Novel Kunitz-type Serine Protease Inhibitor.** J. Biol. Chem. 272:6370-6376, 1997. PubMed: 9045658

Spanjaard RA, Lee PJ, Sarkar S, Goedegebuure PS, Eberlein TJ. **Clone 10d/BM28 (CDCL1), an early s-phase protein, is an important growth regulator of melanoma.** Cancer Res. 57:5122-5128, 1997. PubMed: 9371513

Komatsu M, Carraway CA, Fregien NL, Carraway KL. **Reversible disruption of cell-matrix and cell-cell interactions by overexpression of sialomucin complex**. J. Biol. Chem. 272:33245-33254, 1997. PubMed: 9407114

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ATCC Warranty

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Disclaimers

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

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

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

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Material Safety Data Sheet

Revision Date: 10-Jul-2007

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

Product code C0025C
Product name HEMn-LP, 500,000 cells/vial

Company/Undertaking Identification

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

INVITROGEN CORPORATION
3 FOUNTAIN DRIVE
INCHINNAN BUSINESS PARK
PAISLEY, PA4 9RF
SCOTLAND
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INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
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CANADA L7P 1A1
1-800-263-6236

CASCADE BIOLOGICS
INVITROGEN CORPORATION
1341 S.W. CUSTER DRIVE
PORTLAND, OR 97219
++1 503-292-9521
++1 800-778-4770

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

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

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

3. HAZARDS IDENTIFICATION

3. HAZARDS IDENTIFICATION

Emergency Overview

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

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

Form

Suspension

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

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

Target Organ Effects No information available

HMIS

Health	1
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with soap and plenty of water removing all contaminated clothes and shoes.
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Rinse mouth.
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Water spray. Carbon dioxide (CO2). Foam. Dry powder. alcohol-resistant foam. The product is not flammable.
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. Clean contaminated surface thoroughly. Take up mechanically and collect in suitable container for disposal.

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

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

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

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

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Suspension

Important Health Safety and Environmental Information

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

10. STABILITY AND REACTIVITY

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

11. TOXICOLOGICAL INFORMATION

Acute toxicity

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

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

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

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

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

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

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

15. REGULATORY INFORMATION

International Inventories

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

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

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

U.S. State Regulations

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

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

WHMIS hazard class:
Non-controlled

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

16. OTHER INFORMATION

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

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

End of Safety Data Sheet

SAFETY DATA SHEET

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

Identification of the substance/preparation

Product code C2025C
Product name HEMn-DP, 500,000 cells/vial

Company/Undertaking Identification

Life Technologies
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
+1 760 603 7200

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

24 hour Emergency Response (Transport):

866-536-0631
301-431-8585
Outside of the U.S. +1-301-431-8585

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

2. Hazards identification

GHS - Classification

Signal Word
WARNING

Health Hazard

Skin Corrosion/Irritation	Category 2
Serious Eye Damage/Eye Irritation	Category 2

Physical Hazards
not hazardous

Hazard statements
H315 - Causes skin irritation
H319 - Causes serious eye irritation

Precautionary statements
P264 - Wash hands thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P332 + P313 - If skin irritation occurs: Get medical advice/attention
P337 + P313 - If eye irritation persists: Get medical advice/attention
P362 - Take off contaminated clothing and wash before reuse

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

Carcinogenic effects	none
Mutagenic effects	none
Reproductive toxicity	none
Sensitization	none

Target Organ Effects None under normal use conditions

HMIS

Health	1
Flammability	0
Reactivity	0

3. Composition/information on ingredients

Chemical Name	CAS-No	EINECS-No	Weight %
dimethylsulfoxide	67-68-5	200-664-3	7-13

We recommend handling all chemicals with caution.

4. First aid measures

Skin contact Wash off immediately with plenty of water for at least 15 minutes. Remove and wash contaminated clothing before re-use. Immediate medical attention is required.

Eye contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required.

Ingestion Call a physician or Poison Control Centre immediately. Never give anything by mouth to an unconscious person. Do not induce vomiting without medical advice.

Inhalation Move to fresh air. If not breathing, give artificial respiration. Call a physician or Poison Control Centre immediately.

Notes to physician Treat symptomatically.

5. Fire-fighting measures

Suitable extinguishing media Water spray. Carbon dioxide (CO2). Foam. Dry chemical.

Special protective equipment for firefighters Wear self-contained breathing apparatus and protective suit.

6. Accidental release measures

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

Methods for cleaning up Soak up with inert absorbent material.

Environmental precautions

Prevent further leakage or spillage if safe to do so. Prevent product from entering drains.

See Section 12 for additional information.

7. Handling and storage

Handling Always wear reccommended Personal Protective Equipment. No special handling advice required.

Storage Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers.

8. Exposure controls/personal protection

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 Impervious 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. Do not allow material to contaminate ground water system.

9. Physical and chemical properties

General Information

Form	liquid	
Appearance	No information available	
Odor	No information available	
Boiling Point/Range	**°C** no data available	**°F** no data available
Melting point/range	**°C** no data available	**°F** no data available
Flash point	**°C** no data available	**°F** no data available
Autoignition temperature	**°C** no data available	**°F** no data available
Oxidizing properties	No information available.	
Water solubility	soluble	

10. Stability and reactivity

Stability Stable under normal conditions.
Materials to avoid Acid chlorides. Strong acids Strong oxidizing agents Reducing agents.
Hazardous decomposition products Carbon oxides Sulphur oxides
polymerization None under normal processing.

11. Toxicological information

Acute toxicity

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

Principle Routes of Exposure/ Potential Health effects

Eyes	Irritating to eyes.
Skin	Irritating to skin Components of the product may be absorbed into the body through the skin.
Inhalation	May cause irritation of respiratory tract.
Ingestion	May be harmful if swallowed Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea.
Carcinogenic effects	none
Mutagenic effects	none
Reproductive toxicity	none
Sensitization	none
Target Organ Effects	None under normal use conditions

12. Ecological Information

Ecotoxicity effects	No information available.
Acute aquatic toxicity	Not classified for acute
Chronic aquatic toxicity	Not classified chronic
Mobility	No information available.
Biodegradation	No information available.
Bioaccumulation	No information available.

Chemical Name	Freshwater Algae Data	Water Flea Data	Freshwater Fish Species Data	Microtox Data	log Pow
dimethylsulfoxide 67-68-5	Skeletonema costatum EC5012350 - 25500 mg/L (96 h)	Daphnia species EC50=7000 mg/L (24 h)			logPow-2.03

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	none
Subsidiary Class	none
Packing group	none
UN-No	none

15. Regulatory information

Component	TSCA
dimethylsulfoxide 67-68-5 (7-13)	Listed

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

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

U.S. State Regulations

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

WHMIS hazard class:
D2B Toxic materials

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

16. Other information

Reason for Revision (M)SDS sections updated.

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

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 unkown 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 WARRENTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PUPOSE.

End of Safety Data Sheet

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Material Safety Data Sheet

Revision Date: 10-Jul-2007

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

Product code C0245C
Product name HEMa-LP, 500,000 cells/vial

Company/Undertaking Identification

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

INVITROGEN CORPORATION
3 FOUNTAIN DRIVE
INCHINNAN BUSINESS PARK
PAISLEY, PA4 9RF
SCOTLAND
011 44 141 814 6100

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

CASCADE BIOLOGICS
INVITROGEN CORPORATION
1341 S.W. CUSTER DRIVE
PORTLAND, OR 97219
++1 503-292-9521
++1 800-778-4770

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

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

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

3. HAZARDS IDENTIFICATION

3. HAZARDS IDENTIFICATION

Emergency Overview

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

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

Form

Suspension

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

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

Target Organ Effects No information available

HMIS

Health	1
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with soap and plenty of water removing all contaminated clothes and shoes.
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Rinse mouth.
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Water spray. Carbon dioxide (CO2). Foam. Dry powder. alcohol-resistant foam. The product is not flammable.
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. Clean contaminated surface thoroughly. Take up mechanically and collect in suitable container for disposal.

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

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

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

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

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Suspension

Important Health Safety and Environmental Information

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

10. STABILITY AND REACTIVITY

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

11. TOXICOLOGICAL INFORMATION

Acute toxicity

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

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

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

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

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

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

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

15. REGULATORY INFORMATION

International Inventories

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

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

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

U.S. State Regulations

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

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

WHMIS hazard class:
Non-controlled

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

16. OTHER INFORMATION

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

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

End of Safety Data Sheet

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:	**Dale W Laird**
DEPARTMENT:	**Anatomy and Cell Biology**
ADDRESS:	**DSB 00077**
PHONE NUMBER:	**519 661-2111 x86827**
EMERGENCY PHONE NUMBER(S):	**519 673-3343**
EMAIL:	**dale.laird@schulich.uwo.ca**

Location of experimental work to be carried out :

Building :	**Dental Science Building**	Room(s):	**00077**
Building :	**Dental Science Building**	Room(s):	**00066**
Building :	**Dental Science Building**	Room(s):	**00015, 00017**

***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, CBCF, CRC**

GRANT TITLE(S): **Cx26 and Panx1 as breast tumor suppressors: potential therapeutic targets CBCF**
Cx43 mutations linked to human disease CIHR
Comparative analysis of the life cycle and function of connexins & pannexins CIHR
Functional role of connexins in … CIHR

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
Dale W Laird	**dale.laird@schulich.uwo.ca**	**1997**
Qing (Cindy) Shao	**cindy.shao@schulich.uwo.ca**	**1998, 2002,**

Silvia Penuela	silvia.penuela@schulich.uwo.ca	Oct. 2005
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Shreya Podder	spodder2@uwo.ca	Oct 2010
Wesley Lai	wlai43@uwo.ca	May 2011

April 30/12 Per conversation with C. Shao JS

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.

Cell Lines: All cell lines including viral packaging cells are cultured and passaged in laminar flow hoods that are certified annually for level 2 containment. For long term storage cells are initially flozen in a biohazard labeled -80oC freezer prior to being transferred to a biohazard labeled liquid nitrogen tanks one or two days after freezing. Unwanted cells are bleached and autoclaved prior to diposal. Cells expressing connexins, pannexins or site-directed mutants of these molecules as well as cells ectopically expressing cDNAs are frequently used for Western blots, immunoflourescent or other biochemical analysis.

cDNA constructs: All cDNA constructs(connexins and pannexins) are stored in a -20oC freezer and viral particles are stored in a biohazard labeled -80oC freezer. Reference cell lines or primary cells are transfected or infected with cDNAs to assess the the biochemical and functional proprties of connexins and pannexins. Stably infected or transfected cells are cultured and passaged under level 2 containment. Unwanted transfected or infected cell lines are killed by bleaching , autoclaving, and disposal as biohazardous labeled waste.

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

Most cells in the human body need to consistently talk to each other and exchange information. Cell to cell communication can be mediated by connexin channels called gap junctions that directly allow the exchange of signals. In other cases, information encoded in small molecules are released through specialized channels only to be recaptured by receptors or channels that reside on the surface of neighboring cells. In the present study we are focusing on a new class of channel forming proteins called pannexins that allow small molecules carrying important signals to enter and leave the cell. Already these unique channels have been demonstrated to be important in sending find me signals to clear dead or dying cells. In disease, pannexin channels have been linked to ischemic and epileptic damage in the brain. In addition, our evidence suggests that these channels are very important in skeletal development and skin differentiation. In the present study we will determine what regulates the function of the channels allowing them to release or uptake important signalling molecules. Using newly generated transgenic mouse models that lack one or two members of the pannexin family we will assess the skin and skeleton for abnormalities. Finally, since preliminary data suggests pannexin channels may be important in skeletal diseases, we will determine if the loss of one or more pannexins will lead to the acceleration or reversion of osteoarthritis.

It is a general requirement for normal function that adjacent cells within human tissues communicate directly with each other through special channels called gap junctions. In over 10 distinct human diseases these channels are either not produced by the cells (e.g. many cancers), or the proteins (connexins) that make up these channels contain mutations that inhibit their normal function (e.g. skin diseases, deafness, neuropathies). There are 21 connexin genes in the human genome and multiple connexins can be expressed and intermix in the same cells, leading to a complex array of gap junction channel types within tissues and organs. This is most evident in the epidermis of the skin where up to 10 different connexins are expressed and mutations in anyone of 5 of these connexins result in a variety of human skin diseases. Importantly, mutations in some of these same connexins cause additional disease burden which includes hearing loss and the disease symptoms tend to become evident during aging. In this study we will investigate how connexin mutations cause human diseases of the skin and why these disease manifest as a patient ages. Once it is better understood how connexin mutations cause diseases of the skin in aged patients it is anticipated that these findings could be translated to pre-clinical studies and possible treatments of gap junction-linked diseases.

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:

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
~~***DHS a Ecoli***~~ E. coli dH5 alpha – per email Mar 14, 2012	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**<500 ml**	**Invitrogen**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
E. coli ***JM109***	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**<500 ml**	**Invitrogen**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
Adenovirus		☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
Retrovirus		☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

****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: **Those competent cells are used for tranformation to amplify plasmid DNAs**

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	**skin biopsies**	Not applicable
Rodent	☒ Yes ☐ No	**genetically modified mice, fibroblasts**	**2006-101**
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	**HeLa, HEK293T, HEK, see append,**	**2**	**ATCC**
Rodent	☒ Yes ☐ No	**MDCK, N2A, BICR-M1Rk, see append,**	**2**	**Clonetic, ATCC**
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: **These cell lines have previously been approved and records are on file with the Safety Office. See the appendix for a list of other cell lines in our laboratory.**

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	**ODDD patients and relatives**	☐ Yes ☒ Unknown		☐1 ☒2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or	**ODDD patients**	☐ Yes		☐1 ☒2

April 30/12 - per conversation with C. Shao → Ocular Digital Dental Dysplasia

Tissues (unpreserved)	**and relatives**	☒ Unknown		☐ 2+ ☐ 3
Human Organs or Tissues (preserved)	**ODDD patients and relatives**	Not Applicable		Not Applicable

Additional Comments: **Plans are to expand and seek other patients with connexin-linked diseases**

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?
JM109	**T-East, pcDNA3 (+), (-), pEGFP See appendix**	**Promega, Invitrogen, Clontech**	**connexin, pannexins**	**No**	**No**	**No**

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
Retrovirus Adenovirus	**AP-2 pRNA-H1.1**	**Dr. J. Galipeau, McGill University GenScript**	**connexins, pannexins and N-cadherin**	**improved cell to environment and cell-cell communication**

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
- E1A oncogene ☐ NO ☒ YES
- Known oncogenes ~~☒~~ NO ☒ YES, specify HeLa cells (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 **Mice**

7.3 AUS protocol # **2006-101**

7.4 List the location(s) for the animal experimentation and housing. **West Valley and Dental Science Buildings**

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.

9.3 What is the LD_{50} (specify species) of the toxin or hormone **250ug/kg**

9.4 How much of the toxin or hormone is handled at one time*? **100ng/ml into culture medium**

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

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: **Cholera toxin will be used in MCF10A cell culture medium to facilitate the optimum growth conditions.**

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

Cholera toxin from Cedarlane

12.1 Will any of the above agents be imported? ☒ YES, co
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 # **C-12-1146** ☐ 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 Dale W Laird ***Date:*** March 7, 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: **May 19, 2011**
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-0017**

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:
The infectious material will be washed away if possible, the injured worker will be sent to emergency for assessment, an accident report will be filed and the biosafey office will be contacted to ensure that all necessary accident procedures are executed.

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 **Dale W Laird** ***Date:*** **March 7, 2012**

15.4 Additional Comments: _____

16.0 Approvals

1) UWO Biohazards Subcommittee:
SIGNATURE: _____
Date: May 2 2012

2) Safety Officer for the University of Western Ontario
SIGNATURE: J Stanley
Date: April 30, 2012

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: BIO-UWO-0017 Expiry Date (3 years from Approval): May 2, 2015

Special Conditions of Approval:

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are consider to be level 1 animal pathogens:

- 5K
- 58
- 58-161
- 679
- 1532
- AB284
- AB311
- AB1157
- AB1206
- AG1
- B
- BB4
- BD792
- BL21
- BL21 (DE3)
- BM25.8
- C
- C-1a
- C-3000
- C25
- C41 (DE3)
- C43 (DE3)
- C600
- Cavalli Hfr
- CIE85
- DH1
- DH10 GOLD
- DH10B
- DH5
- DH5-alpha
- DP50
- DY145
- DY380
- E11
- EJ183
- EL250
- EMG2
- EPI 300
- EZ10
- FDA Seattle 1946
- Fusion-Blue
- H1443
- HF4714
- HB101
- HS(PFAMP)R
- Hfr3000
- Hfr3000 X74
- HMS174
- J52
- J53
- JC3272
- JC7661
- JC9387
- JF1504
- JF1508
- JF1509
- JJ055
- JM83
- JM101
- JM109
- K12
- KC8
- KA802
- KAM32
- KAM33
- KAM43
- LE450
- LE451
- LE452
- MB408
- MBX1928
- MC1061
- MC4100 (MuLac)
- MG1655
- MM294
- MS101
- NC-7
- Nissle 1917
- One Shot STBL3
- OP50
- P678
- PA309
- PK-5
- PMC103
- PR13
- Rri
- RV308
- S17-1λ -PIR
- SCS1
- SMR10
- SOLR
- SuperchargeEZ10
- SURE
- TOP10
- TG1
- U5/41
- W208
- W945
- W1485
- W3104
- W3110
- WA704
- WP2
- X1854
- X2160T
- X2541
- X2547T
- XL1-BLUE
- XL1-BLUE-MRF
- XLOLR
- Y10
- Y1090 (1090)
- YN2980
- W3110
- WG1
- WG439
- WG443
- WG445

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

Cinthia Labrie
Head, Animal Pathogen Importation Program
Office of Biohazard Containment & Safety

Canada

Subject: Re: Biological Agents Registry Form: Laird
From: Cindy Shao <Cindy.Shao@schulich.uwo.ca>
Date: 3/14/2012 9:57 AM
To: Jennifer Stanley <jstanle2@uwo.ca>

You are right, it is DH5 alpha. It is competent cells. Cindy

Jennifer Stanley <jstanle2@uwo.ca> 3/13/2012 5:12 PM >>>
Hi there -
I got your form in the mail - thanks.
Just a quick question - do you use DH5 alpha (on the form it says DHS
but I imagine that this is a typo)?
Regards
Jennifer

Laird Biohazard registry Appendix:

Additional grant applications that have been submitted and are pending.

1) **The role of Cx26 and Cx30 in aging skin (CIHR)**
2) **The cellular life and function of pannexins (CIHR)**

Appendix 2.3, Types of established cell lines that are used in our laboratory:

Cell Type and specific cell lines

1. Human: CRL-7762, CRL-7804, CRL-7761, CCD-1074, SC.ZR75, Hs578T, MDA-MB-231, MDA-MB-435S, MCF10A, SK-HEP-1, HBL-100, MCF7, HaCaT, SUM159, SUM149, HeLa, HEK293, 293T
2. Rodent: C2C12, BL6, F0 melanomas, F10 melanomas, L10BIOBR-GFP, NRK, MC3T3-E1, NRK-52E, bEPD0670_4_B09, PC12, N2A, rat epidermal keratinocytes (REK), MDCK, BICR-M1Rk mammary tumor
3. Others: MDCK

All cell lines were obtained from ATCC or colleague laboratories and are handled under level 2 containment although many of these cell lines are designated containment level 1.

Appendix 4.0 Genetically-modified organisms and cell lines:

4.2: Plasmids:

pcDNA-mRFP from Addgene,
pTagRFP vector from Evrogen,
CMV-R-GECO1 from Addgene,
pRc/CMC from Invitrogen,
Tet-ON/off Cherry-vectors set from Clontech.
pEBTet GFP-vector from Germany, Mitochondria-RFP vector from Yale.
pEGFP fusion variants of connexins and pannexins and site-directed or truncated mutants of these genes.

Cell Biology

ATCC® Number: **CRL-7761™** Order this Item

Price: **$459.17 (non-profit list price)**
Log In with customer # to see your price

See New Benefits of ATCC Culture

Designations:	TE 353.Sk
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	fibroblast
Source:	**Organ:** skin **Disease:** normal
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Gender:	female
Comments:	Patient was sister of donor for ATCC CRL-7714. Part of the NBL Cell Line Collection. This cell line is neither produced nor fully characterized by ATCC . We do not guarantee that it will maintain a specific morphology, purity, or any other property upon passage. Please see the NBL Repository description.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

Related Links

- NCBI Entrez Search
- Make a Deposit
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- Material Transfer Agreement New!
- Technical Support
- Related Cell Culture Products

BioProducts

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

BioServices

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

BioStandards

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

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is

Cell Biology

ATCC® Number: **CRL-7804™** Order this Item Price:

Contact Customer Service for pricing and availability

See New Benefits of ATCC Culture

Designations:	Hs 456.Sk
Biosafety Level:	1
Shipped:	flask
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	
Source:	**Organ:** skin **Disease:** normal
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Cytogenetic Analysis:	modal number = 46; range = 44 to 47
Age:	80 years
Gender:	female
Ethnicity:	Caucasian
Comments:	The line was established from apparently normal tissue from a patient who had basal cell carcinoma (see ATCC CRL-7806). Part of the NBL Cell Line Collection. This cell line is neither produced nor fully characterized by ATCC . We do not guarantee that it will maintain a specific morphology, purity, or any other property upon passage. Please see the NBL Repository description. **Note:** This item is distributed only within the 50 United States. It is not available for international distribution.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 is recommended **Medium Renewal:** Every 2 to 3 days Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture

Related Links

- NCBI Entrez Search
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement New!
- Technical Support
- Related Cell Culture Products

BioProducts

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

BioServices

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Cell Biology

ATCC® Number:	**CRL-7762™** Order this Item	Price:	**$459.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	TE 354.T
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	fibroblast 
Source:	**Organ:** skin **Disease:** basal cell carcinoma
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,14 D13S317: 11,14 D16S539: 12 D5S818: 10,13 D7S820: 10 THO1: 6 TPOX: 8,12 vWA: 14,17
Gender:	female
Comments:	The patient was sister of donor for ATCC CRL-7714. Part of the NBL Collection. Unlike other cell lines in the NBL Collection, this item has been fully accessioned by ATCC and is covered by the standard warranty.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Protocol:** Remove medium and rinse with 0.25% trypsin, 0.53 mM

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ATCC® Number:	**CRL-2090™**	Order this Item	Price:	**$551.00 (for-profit list price)** **$459.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	CCD-1074Sk
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	fibroblast
Source:	**Organ:** skin **Disease:** normal **Cell Type:** fibroblast
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Age:	42 years
Gender:	female
Comments:	The line was established from skin taken from normal breast tissue removed at mastectomy. Cells senesce after approximately 26 population doublings.
Propagation:	**ATCC complete growth medium:** Iscove's modified Dulbecco's medium, 90%; fetal bovine serum, 10% **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** Every 3 to 4 days
Subculturing:	Remove spent medium, add fresh 0.25% trypsin, 0.03% EDTA solution, rinse and remove trypsin. Let the culture sit at room temperature (or at 37C) for 2 to 5 minutes. Add fresh medium, aspirate and dispense into new flasks. Subculture every 6 to 8 days.
Preservation:	Culture medium, 95%; DMSO, 5%

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ATCC® Number: **CRL-1500™** Order this Item

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Designations:	ZR-75-1
Depositors:	LW Engel
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial 
Source:	**Organ:** mammary gland; breast **Tissue:** duct **Disease:** ductal carcinoma **Derived from metastatic site:** ascites **Cell Type:** epithelial
Cellular Products:	mucin (apomucin, MUC-1, MUC-2)
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.
Receptors:	estrogen receptor, expressed
Tumorigenic:	Yes

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DNA Profile (STR):
Amelogenin: X
CSF1PO: 10,11
D13S317: 9
D16S539: 11
D5S818: 13
D7S820: 10,11
THO1: 7,9.3
TPOX: 8

Cell Biology

ATCC® Number:	**HTB-126™**	Order this Item	Price:	**$431.00 (for-profit list price)** **$359.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	Hs 578T
Depositors:	AJ Hackett
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial PHOTO
Source:	**Organ:** mammary gland; breast **Disease:** carcinoma
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Receptors:	estrogen receptor, not expressed [1119]
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 13 D13S317: 11 D16S539: 9,12 D5S818: 11 D7S820: 10 THO1: 9,9.3 TPOX: 8 vWA: 17
Cytogenetic Analysis:	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]

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Designations:	MDA-MB-231
Depositors:	R Cailleau
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial PHOTO
Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial
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
Receptors:	epidermal growth factor (EGF), expressed transforming growth factor alpha (TGF alpha), expressed
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X CSF1PO: 12,13 D13S317: 13 D16S539: 12 D5S818: 12 D7S820: 8,9 THO1: 7,9.3 TPOX: 8,9 vWA: 15,18
Cytogenetic Analysis:	The cell line is aneuploid female (modal number = 64, range = 52 to 68), 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.

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ATCC® Number:	**HTB-129™** Order this Item	Price:	**$359.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	MDA-MB-435S
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	spindle shaped 
Source:	**Organ:** previously described as: mammary gland; breast **Disease:** previously described as ductal carcinoma **Derived from metastatic site:** pleural effusion
Cellular Products:	tubulin; actin
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** 1976
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 12 D16S539: 13 D5S818: 12 D7S820: 8,10 THO1: 6,7 TPOX: 8,11 vWA: 16,18
Cytogenetic Analysis:	modal number = 56; range = 55 to 62 The cell line is aneuploid human female (XX), with most chromosome counts in the 55 to 60 range. Normal chromosomes N6, N11, and N22 were absent, while chromosomes N7, N13, N18 and N21 were single. Most of the remainder of normal chromosomes were usually paired, but chromosome N2 was triple. Nineteen marker chromosomes were identified, with most of them formed from structural alterations of the missing copies of the normal

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ATCC® Number:	**CRL-10317™** Order this Item	Price:	**$359.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	MCF 10A
Depositors:	Michigan Cancer Foundation
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial
Source:	**Organ:** mammary gland; breast **Disease:** fibrocystic disease **Cell Type:** epithelial
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** August 22, 1984
Applications:	transfection host
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,12 D13S317: 8,9 D16S539: 11,12 D5S818: 10,13 D7S820: 10,11 THO1: 8,9.3 TPOX: 9,11 vWA: 15,17
Isoenzymes:	AK-1, 1 [23084] ES-D, 1 [23084] G6PD, B [23084] GLO-I, 1-2 [23084] PGM1, 1-2 [23084] PGM3, 1 [23084]
Age:	36 years

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ATCC® Number: **HTB-52™** Order this Item

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Designations:	SK-HEP-1
Depositors:	G Trempe, LJ Old
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial
Source:	**Organ:** liver **Tissue:** ascites **Disease:** adenocarcinoma
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	The cells are distributed for research purposes only. The Memorial Sloan-Kettering Cancer Center releases the line subject to the following: 1.) The cells or their products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of these cells must first be negotiated with The Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.
Isolation:	**Isolation date:** 1971
Applications:	transfection host (Roche Transfection Reagents)
Tumorigenic:	Yes

DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 8,12 D16S539: 12 D5S818: 10,13 D7S820: 8,11 THO1: 7,9 TPOX: 9

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ATCC® Number:	**HTB-22™**	Order this Item	Price:	**$359.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	MCF7
Depositors:	CM McGrath
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial 
Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial
Cellular Products:	insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host
Receptors:	estrogen receptor, expressed
Antigen Expression:	Blood Type O; Rh+

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DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 11 D16S539: 11,12 D5S818: 11,12 D7S820: 8,9 THO1: 6 TPOX: 9,12

Cell Biology

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

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Designations:	HeLa
Depositors:	WF Scherer
Biosafety Level:	2 [Cells contain human papilloma virus]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial 
Source:	**Organ:** cervix **Disease:** adenocarcinoma **Cell Type:** epithelial
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 screening for Escherichia coli strains with invasive potential
Virus Susceptibility:	Human adenovirus 3 Encephalomyocarditis virus Human poliovirus 1 Human poliovirus 2 Human poliovirus 3

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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

Cell Biology

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

Price: **$359.17 (non-profit list price)**
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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*
Morphology:	epithelial 
Source:	**Organ:** embryonic kidney
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	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 transfection host viruscide testing
Receptors:	vitronectin, expressed
Tumorigenic:	YES

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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

Cell Biology

ATCC® Number:	**CRL-11268™** Order this Item	Price:	**$359.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	293T/17 [HEK 293T/17]
Depositors:	Rockefeller Univ.
Biosafety Level:	2 [Cells contain Adeno and SV-40 viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* deposited as human
Morphology:	epithelial
Source:	**Organ:** kidney
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	The line is available with the following restriction: 1. The cell line was deposited at the ATCC by Rockefeller University and is provided for research purposes only. Neither the cell line nor the products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as a service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the cells, or their products, must first be negotiated with Rockefeller University, Office of Technology Transfer, 1230 York Avenue, New York, NY 10065 Attn: Kathleen A. Denis, Associate Vice President Technology Transfer.
Applications:	293T cells were cloned and the clones tested with the pBND and pZAP vectors to obtain a line capable of producing high titers of infectious retrovirus, 293T/17. These cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability.
Antigen Expression:	SV40 T antigen [45408]
DNA Profile (STR):	Amelogenin: X CSF1PO: 11, 12 D13S317: 12, 14 D16S539: 9, 13 D5S818: 8, 9 D7S820: 11 THO1: 7, 9.3

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ATCC® Number: **CRL-1772™** Order this Item

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Designations:	C2C12
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus*
Morphology:	myoblast 
Source:	**Strain:** C3H **Tissue:** muscle **Cell Type:** myoblast;
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
Comments:	This is a subclone (produced by H. Blau, et al) of the mouse myoblast cell line established by D. Yaffe and O. Saxel. The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. Treatment with bone morphogenic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic. Tested and found negative for ectromelia virus (mousepox).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C

Protocol: IMPORTANT - DO NOT ALLOW CULTURES TO BECOME CONFLUENT.
Cultures must not be allowed to become confluent as this will deplete the myoblastic population in the culture.
Myotube formation is enhanced when the medium is supplemented with 10% horse serum instead of fetal bovine serum.

1. Remove and discard culture medium.

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ATCC® Number:	**CRL-6322™** Order this Item	Price:	**$359.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	B16-F0
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus*
Morphology:	mixture of spindle-shaped and epithelial-like cells
Source:	**Organ:** skin **Strain:** C57BL/6J **Disease:** melanoma
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Roche Transfection Reagents)
Tumorigenic:	Yes
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

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Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is

Cell Biology

ATCC® Number:	**CRL-6475™** Order this Item	Price:	**$359.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	B16-F10
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus*
Morphology:	mixture of spindle-shaped and epithelial-like cells 
Source:	**Organ:** skin **Strain:** C57BL/6J **Disease:** melanoma
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
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is

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Product	L10BIOBR-GFP
Company	American Type Culture Collection (ATCC)
Price	REQUEST INFO Request Information for this Product Pricing Info
More Information	View Company Product Page
Catalog Number	CRL-2770
Quantity	1 vial

Description

The L10BIOBR-GFP cell line was derived by infecting the immortalized murine melanocyte cell line L10BIOBR with pDIVA-GFP. The vector contains the puromycin resistant gene. The cells were selected on medium containing puromycin. Both cell lines, L10BIOBR-GFP (CRL-2770?) and L10BIOBR-MAPKK (ATCC CRL-2771?) will serve as controls for oncogenic transformation and signal transduction studies for melanoma [PubMed: 12514183].

Cell Biology

ATCC® Number:	**CRL-6509™** Order this Item	Price:	**$459.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	NRK
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Rattus norvegicus*
Morphology:	epithelial
Source:	**Organ:** kidney **Strain:** Osborne-Mendel **Disease:** normal
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Roche Transfection Reagents)
Cytogenetic Analysis:	modal number = 44; range = 39 to 44
Age:	adult
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Remove medium, and rinse with 0.25% trypsin, 0.53 mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. **Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:12 is recommended **Medium Renewal:** Every 2 to 3 days

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Cell Biology

ATCC® Number:	**CRL-2593™** Order this Item	Price:	**$431.00 (for-profit list price)** **$359.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	MC3T3-E1 Subclone 4
Depositors:	RT Franceschi
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus*
Morphology:	fibroblast
Source:	**Organ:** bone **Strain:** C57BL/6 **Tissue:** calvaria **Cell Type:** preosteoblast;
Cellular Products:	collagen [51540]
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:	These cell lines are good models for studying in vitro osteoblast differentiation, particularly ECM signaling. They have behavior similar to primary calvarial osteoblasts. The MC3T3-E1 Subclone 4 (ATCC CRL-2593) and the MC3T3 Subclone 14 (ATCC CRL-2594) lines exhibit high levels of osteoblast differentiation after growth in ascorbic acid and 3 to 4 mM inorganic phosphate.
Tumorigenic:	Yes
Age:	newborn

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A series of subclones were isolated from the cloned but phenotypically heterogeneous MC3T3-E1 cell line. The subclones were selected for high or low osteoblast differentiation and mineralization after growth in medium containing ascorbic acid. The MC3T3-E1 Subclone 4 (ATCC CRL-2593) and the MC3T3 Subclone 14 (ATCC CRL-2594) lines exhibit high levels of osteoblast differentiation after growth in ascorbic acid and 3 to 4 mM inorganic phosphate. They form a well mineralized extracellular

Cell Biology

ATCC® Number:	**CRL-1571™** Order this Item	Price:	**$431.00 (for-profit list price)** **$359.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	NRK-52E
Depositors:	JE DeLarco
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Rattus norvegicus* deposited as *Rattus* sp.
Morphology:	epithelial
Source:	**Organ:** kidney **Disease:** normal
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host
Receptors:	epidermal growth factor (EGF); multiplication stimulating activity (MSA)
Propagation:	**ATCC complete growth medium:** Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose, 95%; bovine calf serum, 5% **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%
Subculturing:	**Protocol:** Remove medium, and rinse with 0.25% trypsin, 0.02% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Centrifuge the cell suspension at 1000 rpm for 10 minutes, resuspend the pellet in fresh culture medium, aspirate and dispense into new culture vessels. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:4 is recommended

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ATCC® Number:	**CRL-1721™**	Order this Item	Price:	**$431.00 (for-profit list price)** **$359.17 (non-profit list price)** **Log In with customer # to see your price**

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Designations:	PC-12
Depositors:	B Patterson
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	floating clusters; few scattered lightly attached cells.
Organism:	*Rattus norvegicus* deposited as *Rattus* sp.
Morphology:	small irregularly shaped cells PHOTO
Source:	**Organ:** adrenal gland **Disease:** pheochromocytoma
Cellular Products:	catecholamines; dopamine; norepinephrine
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
Receptors:	nerve growth factor (NGF), expressed
Tumorigenic:	Yes
Cytogenetic Analysis:	40 chromosomes; 38 autosomes plus XY [1163]
Gender:	male
Comments:	The PC-12 cell line was derived from a transplantable rat pheochromocytoma. The cells respond reversibly to NGF by induction of the neuronal phenotype when plated on Collagen IV coated culture flasks. The cells do not synthesize epinephrine.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: • heat-inactivated horse serum to a final concentration of 10% • fetal bovine serum to a final concentration of 5%

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ATCC® Number:	**CCL-131™** Order this Item	Price:	**$431.00 (for-profit list price)** **$359.17 (non-profit list price)** **Log In with customer # to see your price**

See New Benefits of ATCC Culture

Designations:	Neuro-2a
Depositors:	RJ Klebe
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus*
Morphology:	neuronal and amoeboid stem cells PHOTO
Source:	**Organ:** brain **Strain:** A **Disease:** neuroblastoma **Cell Type:** neuroblast;
Cellular Products:	acetylcholinesterase tubulin
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
Virus Susceptibility:	Herpes simplex virus Vesicular stomatitis virus Human poliovirus 1
Antigen Expression:	H-2, a haplotype; Mus musculus, expressed
Cytogenetic Analysis:	modal number = 95; range = 59 to 193. Karyotype unstable within a stemline range of 94 to 98 chromosomes. All the cells contain 6 to 10 large chromosomes with median or submedian centromeres and 2 to 4 minute chromosomes.
GenoType:	albino
Comments:	Clone Neuro-2a was established by R.J. Klebe and F.H. Ruddle from a spontaneous tumor of a strain A albino mouse. This tumor line, designated C1300, was obtained from the Jackson Laboratory, Bar Harbor, Maine [22161]. Neuro-2a cells produce large quantities of microtubular protein which is believed to play a role in a contractile system which is responsible for axoplasmic flow in nerve cells. The cell line has been used for studies on the mechanism of vinblastine precipitation of microtubular protein, the kinetics of

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ATCC® Number: **CCL-34™** Order this Item

Price: **$359.17 (non-profit list price)**
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Designations:	MDCK (NBL-2)
Depositors:	S Madin, NB Darby
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Canis familiaris*
Morphology:	epithelial 
Source:	**Organ:** kidney **Disease:** normal
Cellular Products:	keratin
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** September, 1958
Applications:	transfection host
Virus Susceptibility:	Human Coxsackievirus B 5 Reovirus type 2 Adeno-associated virus 4 Vaccinia virus Vesicular stomatitis virus Adeno-associated virus 5 Human Coxsackievirus B 3 Human Coxsackievirus B 4 Human poliovirus 2
Cytogenetic Analysis:	Polyploidy 0.2%. Two large submetacentric chromosomes noted, presumably X chromosomes, and one or two additional chromosomes with median or submedian centromeres.
Age:	adult
Gender:	female
Comments:	The MDCK cell line was derived from a kidney of an apparently normal adult female cocker spaniel, September, 1958, by S.H. Madin and N.B. Darby. The cells are positive for keratin by immunoperoxidase staining. MDCK cells have been used to study processing of beta amyloid precursor protein and sorting of its

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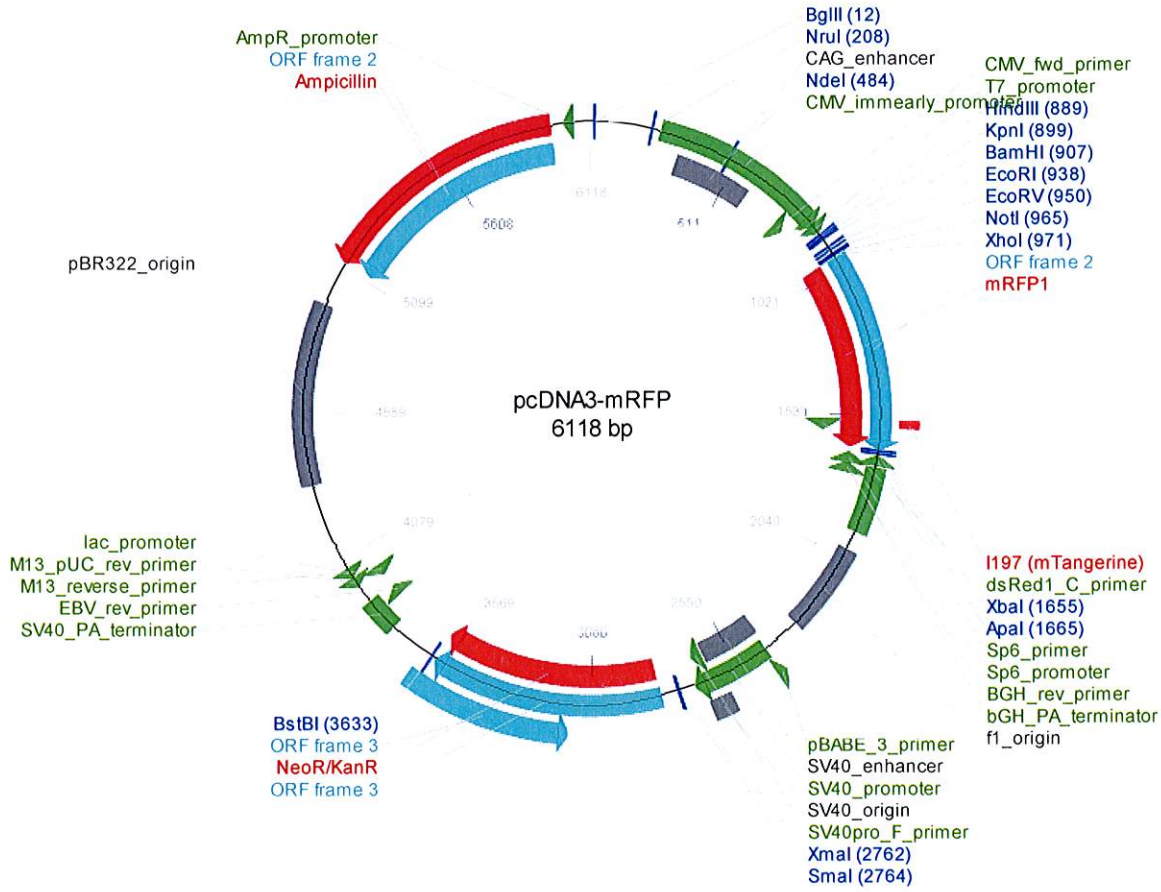
A better way to share plasmids

Plasmid 13032: pcDNA3-mRFP

Gene/insert name:	Monomer Red Fluorescent Protein
Alt name:	mRFP
Insert size:	678
GenBank ID:	AF506027
Vector backbone:	pcDNA3 (Search Vector Database)
Backbone manufacturer:	Invitrogen
Vector type:	Mammalian Expression
Backbone size w/o insert:	5446
Cloning site 5':	XhoI
Site destroyed during cloning:	No
Cloning site 3':	XbaI
Site destroyed during cloning:	No
5' sequencing primer:	T7 List of Sequencing Primers
3' sequencing primer:	GTCTTGTAGTTGCCGTCGTC
Bacterial resistance:	Ampicillin
Growth strain:	DH5alpha
Growth temperature (°C):	37
High or low copy:	High Copy
Selectable markers:	Neomycin
Person or lab that originally cloned the gene/insert:	mRFP is from Roger Tsien, UCSD.
Sequence:	View sequences (2)
Principal Investigator:	Doug Golenbock
Terms and Licenses:	MTA Clontech Limited Use Label License

Comments: mRFP cloned into the old, discontinued pcDNA3 vector from Invitrogen.

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.

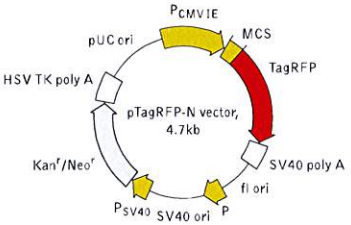


Feature Name	Start	End
CMV_immearly_promoter	236	852
CAG_enhancer	315	602
CMV_fwd_primer	769	789
T7_promoter	863	881
mRFP1	980	1651
I197 (mTangerine)	1553	1579
dsRed1_C_primer	1569	1592
Sp6_primer	1688	1671
Sp6_promoter	1689	1672
BGH_rev_primer	1725	1708
bGH_PA_terminator	1711	1938
f1_origin	2001	2307
pBABE_3_primer	2441	2421
SV40_enhancer	2643	2427
SV40_promoter	2439	2708
SV40_origin	2607	2684
SV40pro_F_primer	2669	2688
NeoR/KanR	2826	3614
SV40_PA_terminator	3794	3913
EBV_rev_primer	3882	3901
M13_reverse_primer	3975	3957
M13_pUC_rev_primer	3996	3974
lac_promoter	4039	4010
pBR322_origin	4967	4348
Ampicillin	5982	5122
AmpR_promoter	6052	6024

evrogen

pTagRFP-N vector

The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by Evrogen. This vector has not been completely sequenced.



For vector sequence, please visit our Web site at http://www.evrogen.com/support/vector-info.shtml

Product	Cat.#	Size
pTagRFP-N vector	**FP142**	20 μg

The price does not include delivery. The price varies in different countries. Please contact your local distributor for exact prices and delivery information.

Vector type	mammalian expression vector
Reporter	TagRFP
Reporter codon usage	mammalian
Promoter for TagRFP	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	TagRFP expression in mammalian cells; generation of fusions to the TagRFP N-terminus

Multiple cloning site (MCS)

Nhe I — Bgl II — Sac I — EcoR I — Sal I — Sac II — Sma I/Xma I — Age I — TagRFP

...G.CTA.GCG.CTA.CCG.GAC.TCA.GAT.CTC.GAG.CTC.AAG.CTT.CGA.ATT.CTG.CAG.TCG.ACG.GTA.CCG.CGG.GCC.CGG.GAT.CCA.CCG.GTC.GCC.ACC.ATG.G...

Afe I — Xho I — Hind III — Pst I — Kpn I — Apa I — BamH I — Nco I*

* — not unique site.

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
MCS: 591-671
TagRFP
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681; Stop codon: 1390-1392
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1542-1547 & 1571-1576
mRNA 3' ends: 1580 & 1592
f1 single-strand DNA origin: 1639-2094
Eukaryotic promoter for expression of Kan^r gene
-35 region: 2156-2161; -10 region: 2179-2184
Transcription start point: 2191
SV40 origin of replication: 2435-2570
SV40 early promoter
Enhancer (72-bp tandem repeats): 2268-2339 & 2340-2411
21-bp repeats: 2415-2435, 2436-2456 & 2458-2478
Early promoter element: 2491-2497
Major transcription start points: 2487, 2525, 2531 & 2536
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2619-2621; Stop codon: 3411-3413
G->A mutation to remove Pst I site: 2801
C->A (Arg to Ser) mutation to remove BssH II site: 3147
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3649-3654 & 3662-3667
pUC plasmid replication origin: 3998-4641

References

Gorman (1985). "High efficiency gene transfer into mammalian cells." In: *DNA cloning: A Practical Approach, Vol. II.* Ed. by Glover. (IRL Press, Oxford, U.K.) Pp. 143–190.

Haas et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." Curr Biol, 6 (3): 315–324 / pmid: 8805248

Kozak (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." Nucleic Acids Res, 15 (20): 8125–8148 / pmid: 3313277

Vector description

pTagRFP-N is a mammalian expression vector encoding red (orange) fluorescent protein TagRFP. The vector allows generation of fusions to the TagRFP N-terminus and expression of TagRFP fusions or TagRFP alone in eukaryotic (mammalian) cells.

TagRFP codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TagRFP sequence [Kozak 1987]. Multiple cloning site (MCS) is located between $P_{CMV\ IE}$ and TagRFP coding sequence.

The vector backbone contains immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in *E. coli* and f1 origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3'-end of the reporter mRNA.

SV40 early promoter (P_{SV40}) provides neomycin resistance gene (Neo^r) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan^r) in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Generation of TagRFP-tagged fusions

A localization signal or a gene of interest should be cloned into MCS of the vector. It will be expressed as a fusion to the TagRFP N-terminus when inserted in the same reading frame as TagRFP and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. TagRFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express TagRFP, when transfected into eukaryotic (mammalian) cells.

Note: The plasmid DNA was isolated from dam$^+$-methylated *E.coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam$^-$ host and make fresh DNA.

Expression in mammalian cells

pTagRFP-N vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 [Gorman 1985].

Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 μg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

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Evrogen Fluorescent Protein Products (the Products) are intended for research use only. The Products are covered by U.S. Pat. 7,417,131 and other Evrogen Patents and/or Patent applications pending. By use of these Products, you accept the terms and conditions of the applicable Limited Use Label License.

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MATERIAL SAFETY DATA SHEET INFORMATION: To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.

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A better way to share plasmids

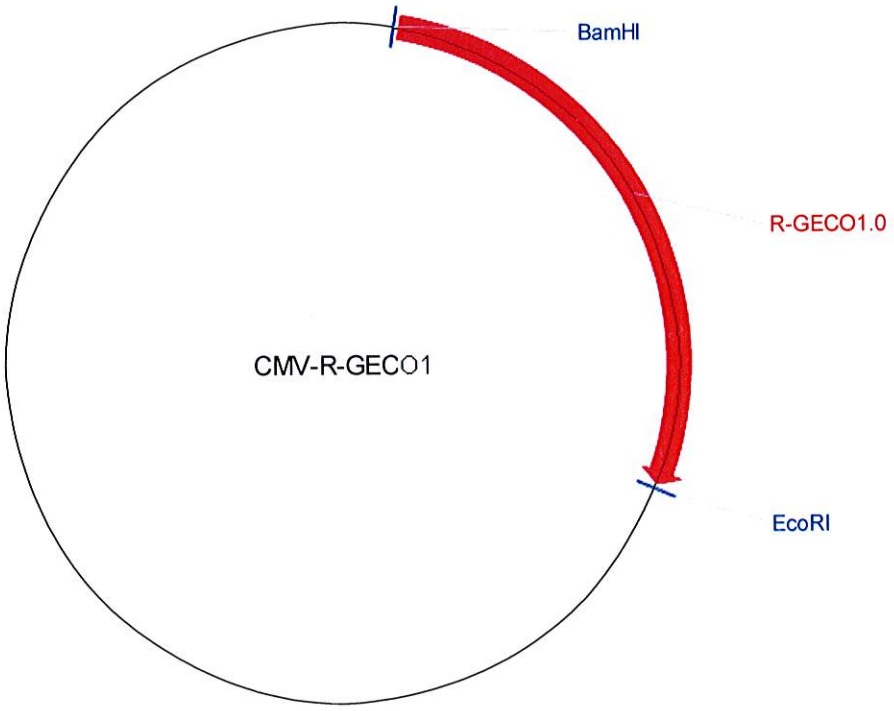
Browse > Robert Campbell > Zhao et al > CMV-R-GECO1

Plasmid 32444: CMV-R-GECO1

Gene/insert name:	R-GECO1.0
Alt name:	red intensiometric genetically encoded Ca2+-indicators for optical imaging
Insert size:	1254
Species:	synthetic construct
GenBank ID:	JN258411
Mutation:	Substitutions relative to the mApple-derived analogue of GCaMP3: T47A/L60P/E61V/S63V/E64S/R81G/K83R/Y134C/M158L/N164aD/V228A/S290P/I366F/K380N/S404G/N414D/E430V
Vector backbone:	Customized Vector (Search Vector Database)
Vector type:	Mammalian Expression
Backbone size w/o insert:	3200
Promoter:	CMV
Cloning site 5':	BamHI
Site destroyed during cloning:	No
Cloning site 3':	EcoRI
Site destroyed during cloning:	No
5' sequencing primer:	TAATACGACTCACTATAGGG List of Sequencing Primers
3' sequencing primer:	TAGAAGGCACAGTCGAGG
Bacterial resistance:	Ampicillin
Growth strain:	DH10B
Growth temperature (°C):	37
High or low copy:	High Copy
Sequence:	View sequences (3)
Principal Investigator:	Robert Campbell
Terms and Licenses:	MTA

Comments: Note: Could not make stable cell line using this vector.

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.



Article: An Expanded Palette of Genetically Encoded Ca2+ Indicators. Zhao et al (Science. 2011 Sep 8. PubMed)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Addgene plasmid 32444" in your Materials and Methods section.

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addgene **Vector Database**

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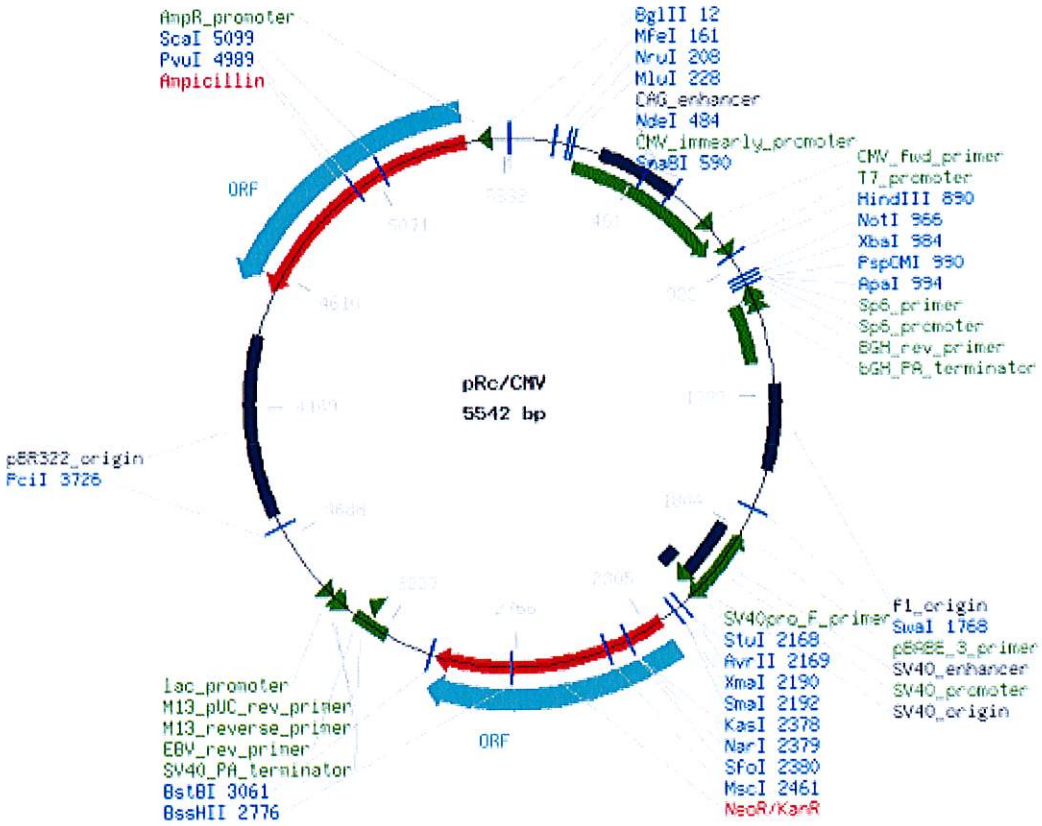
Plasmid Name	pRc/CMV
Alt Names	RcCMV
Source/Vendor	Invitrogen
Plasmid Type	Mammalian
Promoter	CMV
Plasmid Size	5542
Sequencing Primer	T7/Sp6
Bacterial Resistance	Amp
Mammalian Selection	Neomycin
Catalog Number	V75020
Plasmid Sequence	View Sequence


AmpR_promoter
ScaI 5099
PvuI 4989
Ampicillin
ORF
BglII 12
MfeI 161
NruI 208
MluI 228
CAG_enhancer
NdeI 484
CMV_immearly_promoter
SnaBI 590
CMV_fwd_primer
T7_promoter
HindIII 890
NotI 966
XbaI 984
PspOMI 990
ApaI 994
Sp6_primer
Sp6_promoter
BGH_rev_primer
bGH_PA_terminator
pRc/CMV
5542 bp
pBR322_origin
PciI 3726
lac_promoter
M13_pUC_rev_primer
M13_reverse_primer
E8V_rev_primer
SV40_PA_terminator
BstBI 3061
BssHII 2776
ORF
SV40pro_F_primer
StuI 2168
AvrII 2169
XmaI 2190
SmaI 2192
KasI 2378
NarI 2379
SfoI 2380
MscI 2461
NeoR/KanR
f1_origin
SwaI 1768
pBABE_3_primer
SV40_enhancer
SV40_promoter
SV40_origin

Tet-On 3G Tetracycline-Inducible Expression Systems

The Tet-On 3G Tetracycline-Inducible Gene Expression Systems are the 3rd generation of the most powerful, versatile, and widely cited inducible mammalian expression systems available. The Tet-On 3G system offers a significant improvement over Tet-On and Tet-On Advanced with significantly **reduced basal expression** and **increased sensitivity** to doxycycline, a tetracycline analogue.

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Mutations within the P_{TRE3G} promoter have reduced background expression from this tetracycline inducible promoter by 5–20-fold compared to our previous tightest promoter, P_{Tight}.

Highest Sensitivity

Mutations to create the Tet-On 3G transactivator have significantly increased its sensitivity to the inducer doxycycline (Dox).

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Fold induction refers to the difference between the induced and uninduced states. Since Tet-On 3G retains the high maximal expression demonstrated by all Tet-On and Tet-Off systems but has significantly reduced basal expression compared to its predecessors, the fold induction levels are far higher.

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Fast set-up of doxycycline-inducible protein expression in human cell lines with a single plasmid based on Epstein–Barr virus replication and the simple tetracycline repressor

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We have developed a novel plasmid vector, pEBTetD, for full establishment of doxycycline-inducible protein expression by just a single transfection. pEBTetD contains an Epstein–Barr virus origin of replication for stable and efficient episomal propagation in human cell lines, a cassette for continuous expression of the simple tetracycline repressor, and a cytomegalovirus-type 2 tetracycline operator (tetO2)-tetO2 promoter. As there is no integration of vector into the genome, clonal isolation of transfected cells is not necessary. Cells are thus ready for use 1 week after transfection; this contrasts with 3–12 weeks for other systems. Adequate regulation of protein expression was accomplished by abrogation of mRNA polyadenylation. In northern analysis of seven cDNAs coding for transport proteins, pools of transfected human embryonic kidney 293 cells showed on/off mRNA ratios in the order of 100 : 1. Cell pools were also analyzed for regulation of protein function. With two transport proteins of the plasma membrane, the on/off activity ratios were 24 : 1 and 34 : 1, respectively. With enhanced green fluorescent protein, a 23 : 1 ratio was observed based on fluorescence intensity data from flow cytometry. The unique advantage of our system rests on the unmodified tetracycline repressor, which is less likely, by relocation upon binding of doxycycline, to cause cellular disturbances than chimera of tetracycline repressor and eukaryotic transactivation domains. Thus, in a comprehensive comparison of on- and off-states, a steady cellular background is provided. Finally, in contrast to a system based on Flp recombinase, the set-up of our system is inherently reliable.

The function of human proteins is commonly analyzed by heterologous expression in cultured cell lines. Regulated expression, i.e. a system to switch on expression on demand, has clear advantages over constitutive expression. With constitutive expression, cells may die during antibiotic selection because of toxic effects of the expressed protein [1]. Also, for a close match of backgrounds, it is better to compare two states of a single cell line rather than two separately transfected and selected cell lines.

Several widely used systems for regulated expression in mammalian cell lines are based on the tetracycline

Abbreviations
CMV, cytomegalovirus; EBV, Epstein–Barr virus; ETTh, ergothioneine transporter from human; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; eGFP, enhanced green fluorescent protein; MPP^+, 1-methyl-4-phenylpyridinium; rtTA, reverse tetracycline-controlled transcriptional activator; *tetO2*, type 2 tetracycline operator; TetR, tetracycline repressor; tTA, tetracycline-controlled transcriptional activator; tTS, tetracycline-controlled transcriptional silencer.

repressor (TetR) [2,3]. Current systems require two or three rounds of transfection of separate plasmids and clonal isolation, which makes setting-up an inducible cell line a protracted (at least 3 weeks if one buys cell lines prepared for the final round, or 12 weeks if one starts from scratch) and expensive procedure. In order to avoid clonal selection in the final round of transfection, the Flp-In™-T-Rex™ system (Invitrogen, Karlsruhe, Germany) may be used. Here, in the first transfection, a Flp recombinase target site is introduced randomly into the genome; *tetR* follows in the second transfection. In the final transfection, Flp recombinase from a cotransfected plasmid is used to integrate the plasmid for protein expression into the target site. Since the open reading frame for hygromycin resistance on the expression plasmid lacks a start codon, random integration into the genome does not yield resistant cells. This leads to a uniform pool of transfected cells; clonal selection is unnecessary. Unfortunately, despite intensive scrutiny, we and others have experienced a high failure rate (~90% of all transfections) with this system, where no clones at all were generated in the end, even with the positive control plasmid supplied.

The major bottleneck in stable transfection of cells arises from the low frequency of stable plasmid integration into genomic DNA. At best, only ~0.001% of cells generate clones. Expansion of the few survivors takes weeks, particularly with selection and functional testing of individual clones. In contrast, plasmids with an Epstein–Barr virus (EBV) origin of replication *oriP* in the presence of EBV-encoded EBNA-1 protein are continuously propagated in ~1% of initially transfected cells [4]. EBV plasmid replication has been demonstrated for a large variety of human cell lines; primate and canine cell lines may also be used [5]. It has been extensively documented that the plasmids are maintained episomally (5–10 copies per cell, e.g. for 293 cells), i.e. they do not integrate into genomic DNA [5–7]. Hence, it is expected that clone-specific effects of the genetic neighbourhood (positional effects) on protein expression are avoided [8]. It thus becomes feasible to work with transfected cell pools instead of single cell clones. Altogether, it would save much time to employ an EBV vector that carries all elements necessary for doxycycline-regulated gene expression on a single plasmid.

We have recently developed a substrate search strategy for integral membrane transport proteins termed 'LC-MS difference shading' [9]. Our strategy is based on comparative analysis of lysates of cells both with and without transporter expression. The expression of all other proteins in the two cell populations should match as closely as possible. Thus, for us, a suitable system for regulated gene expression must provide an identical background.

EBV-derived single-plasmid systems for tetracycline-regulated gene expression have been described previously; these are based on the TetR-VP16 fusion proteins tetracycline-controlled transcriptional activator (tTA) [10] or reverse tetracycline-controlled transcriptional activator (rtTA) [8]. A third system [11] is based on concomitant expression of two fusion proteins, i.e. $rtTA2^S$-M2, which contains three tandem repeat VP16 minimal activation domains, and tetracycline-controlled transcriptional silencer $(tTS)^{KRAB}$, which contains the N-terminus of the KRAB repressor domain of the mammalian Kox1 protein. However, it is well known that transactivator domains, such as VP16, interact with a variety of transcription factors [12,13]. Indeed, analysis of expression levels in stably transfected HeLa cells suggests that in high numbers, even TetR fusion proteins based on VP16 minimal activation domains are toxic [12]. Thus, relocation of TetR fusion proteins upon binding of inducer can be expected to cause secondary background differences. Pronounced alteration of rtTA expression levels after addition of inducer would promote further differences [8]. Another single-plasmid EBV system based on regulation by temperature shift (29 °C versus 37 °C) was also expected to display disturbing background differences [14]. Instead we opted to utilize continuous expression of the unmodified TetR. The original tetracycline repressor simply binds to a tandem of the type 2 tetracycline operator (*tetO2*) operator and thus blocks transcription from the upstream cytomegalovirus (CMV) promoter [15]. Addition of tetracycline or doxycycline to the culture medium turns on expression: the inducer binds to the repressor, which then dissociates from the operator. From the lack of interaction of the unmodified TetR with mammalian transcription factors, a steady background can be expected. In addition, evidence from yeast suggests that the inducer doxycycline itself has no significant effect on global transcription levels [16]. Thus, it was our aim to develop a single-plasmid EBV vector for doxycycline-regulated gene expression based on the simple TetR. Such a vector has not been reported before.

Results and Discussion

We have constructed plasmid pEBTet which links all elements necessary for doxycycline-inducible expression with the EBV origin of replication (Fig. 1). The orientation of elements in this vector appears to be critical, since an otherwise identical plasmid with the *tetR* cassette in reverse orientation yielded no viable

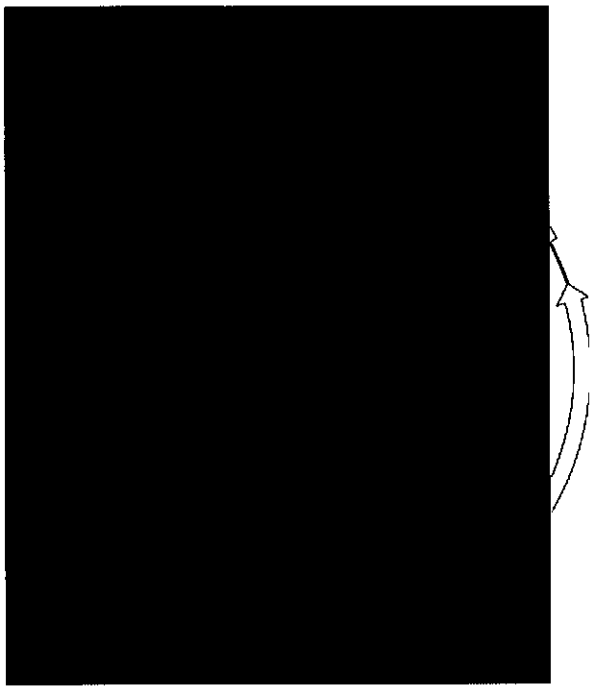

Fig. 1. Plasmid map of pEBTet. The backbone (from oriP clockwise to the puromycin resistance cassette) stems from pCEP-Pu (see Experimental procedures). pEBTetD (11.2 kb) lacks the bovine growth hormone poly(A) site, but is otherwise identical.

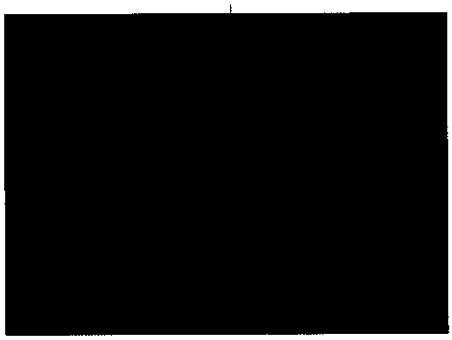

Fig. 2. Northern analysis: regulation of transcription with pEBTet/OCT1h and pEBTetD/OCT1h. mRNA was isolated from stably transfected 293 cell pools that had been cultured with or without 1 $\mu g \cdot mL^{-1}$ doxycycline for 20 h. The RNA blot was first hybridized with a human OCT1 probe. Without stripping, the blot was then hybridized with a GAPDH probe to determine RNA loading.

cells after transfection and antibiotic selection (not shown).

Initially we tried to partition all elements to two plasmids in order to avoid a single large plasmid. However, with the two-plasmid system, where both plasmids contained *oriP* and a different selection marker, growth of transfected cells was unsteady, perhaps because of *oriP* interference. Actually, our worries over the relatively large pEBTet vector (11.5 kb) were unfounded, as standard cloning procedures can be followed.

With pEBTet, the phase of antibiotic selection and cell expansion is much shorter than in other systems outlined above. It takes only about a week after transfection until it is possible to harvest a fully grown culture flask (175 cm^2). For all subsequent analysis, pooled cells were used. With seven cDNAs coding for transport proteins, northern blot analysis of 293 cells transfected with the respective pEBTet plasmids consistently revealed strong transcription in the on-state (= 100%) and low background (1–2%, measured by radioluminography) in the off-state (Fig. 2; Table 1). We have observed comparable ratios (100 : 1) with the Flp-In™-T-Rex™ system. Thus, as far as regulation of transcription is concerned, the pEBTet vector works well: in the off-state, *tetO2* elements are sufficiently covered by TetR to block transcription almost completely.

Transporter expression in 293 cells was assayed functionally by initial rates of uptake of substrates; initial rates of uptake are directly proportional to transporter number. With the ergothioneine transporter from human (ETTh; see Fig. 3A) and enhanced green fluorescent protein (eGFP) chimeras of ETTh and of ORCTL3h (not shown), the high signal-to-noise ratio of the mRNA corresponded to a similar ratio for transport function or eGFP fluorescence intensity. However, with OCT1h (Fig. 3B) and OCT2h (not shown) we obtained inadequate ratios; for our assays, we aim for at least a 10 : 1 ratio. It was reasoned that with some cDNAs, even the low mRNA levels in the off-state generate, because of highly efficient translation, considerable amounts of protein. A 100-fold increase in mRNA level does not increase protein in an equivalent proportion because of the limited capacity of the synthesis machinery, especially for membrane proteins [17]. To improve the signal-to-noise ratio, we thus aimed to reduce the efficiency of translation. In our first attempt, we constructed three variants of pEBTet with hairpins of graded stability (ΔG = −26, −33, or −40 $kcal \cdot mol^{-1}$, calculated with MFOLD [18] version 3.2; 1 cal = 4.184 J) in the 5′-untranslated region downstream of the tandem *tetO2* element. The hairpins were intended to block ribosome progression [19]. Unfortunately, even with the most stable hairpin, the functional signal-to-noise ratio was only slightly improved (not shown).

Our second attempt was based on the notion that the number of translations per mRNA molecule may

Table 1. Regulation of transcription of transporter cDNAs with pEBTet and pEBTetD vectors. For each construct, mRNA was isolated from stably transfected 293 cell pools that had been cultured either with or without 1 $\mu g \cdot mL^{-1}$ doxycycline for 20 h. mRNA was quantitated by northern analysis with radiolabelled probes followed by radioluminography. RNA blots were successively analyzed with a transporter probe and with a GAPDH probe (cf. Fig. 2). Relative background transcription was calculated from the ratios of signals for transporter mRNA and GAPDH mRNA. With OCT1, OCT2, and CAT4 both vectors were analyzed alongside on a single blot. OCT, organic cation transporter; CAT, cationic amino acid transporter.

Transporter cDNA (human)		Vector		Transporter mRNA/GAPDH mRNA		Relative background transcription (%)
Name	Gene symbol			Doxycycline +	Doxycycline –	
ETT	*SLC22A4*	pEBTet		0.014	1.4	1.0
ORCTL3	*SLC22A13*	pEBTet		0.014	1.6	0.9
FLIPT1	*SLC22A16*	pEBTet		0.0011	0.096	1.1
EMT	*SLC22A3*	pEBTet		0.0052	0.33	1.6
OCT1	*SLC22A1*	pEBTet		0.045	1.9	2.3
			pEBTetD	0.0017	0.22	0.8
OCT2	*SLC22A2*	pEBTet		0.068	3.4	2.0
			pEBTetD	0.0077	0.44	1.7
CAT4	*SLC7A4*	pEBTet		0.031	4.9	0.6
			pEBTetD	0.012	1.5	0.8

be influenced by the 3′ end [20,21]. We thus deleted the bovine growth hormone polyadenylation site downstream from the cDNA of interest (Fig. 1) to generate plasmid pEBTetD (11.2 kb). The corresponding mRNA will then lack a poly(A) tail, which is a major stabilizing factor. Conversely, without a polyadenylation site and thus without endonucleolytic cleavage, the mRNA could become much longer, which would increase stability. However, it became evident from northern analysis (Fig. 2) that *oriP*, which contains 24 EBNA-1 binding sites and a 65 base dyad symmetry element [22], can function as a terminator region of RNA polymerase II. Close inspection revealed that the mRNA of OCT1h was predominantly terminated upstream of the EBNA-1 binding site region. With pEBTetD, the copy number of OCT1h mRNA was reduced as compared with pEBTet by a factor of nine in the on-state and by a factor of 26 in the off-state (Table 1).

With pEBTetD, we obtained a high functional signal-to-noise ratio for OCT1h (Fig. 3B). For ETTh, regulation of expression was improved to Flp-In™-T-Rex™ system values (Fig. 3A). After 10 weeks of continuous cell culture, the on/off activity ratio was still maintained for ETT (not shown). Figure 4 shows results from analysis of eGFP expression by flow cytometry. With pEBTetD/eGFP-transfected cells the fluorescence intensity in the off-state (median 6.5) was slightly higher than autofluorescence from nontransfected cells (3.0); in the on-state, median fluorescence intensity was strongly increased (84.3; this amounts to stimulation by a factor of 23). By comparison, with pEBTet/eGFP-transfected cells, the median fluorescence intensity was 195 in the off-state and 1860 in the on-state (this amounts to stimulation by a factor of 9.7). Thus, the background in the off-state was much lower with pEBTetD than with pEBTet. However, in contrast to expression of membrane proteins, the level of expression of cytosolic eGFP in the on-state was higher with pEBTet. It follows that pEBTetD provides low background and moderate expression levels. pEBTet offers very high expression, but the background levels, depending on the cDNA, may be intolerable.

It should be noted that with most cDNAs, dishes of pEBTet-transfected cells showed two- to three-fold reduced protein content after culture with 1 $\mu g \cdot mL^{-1}$ doxycycline for 20 h versus noninduced control cells. No such differences were observed with pEBTetD. It would thus seem that the large amount of polyadenylated mRNA generated in the on-state from pEBTet can impair cell proliferation or viability.

The flow cytometry data for both pEBTet and pEBTetD show a considerable spread in fluorescence intensity; this has also been observed with other expression systems [11]. It remains to be seen whether stably transfected single cell clones can have much higher factors of inducibility than those calculated above for the pools. Bornkamm *et al.* [11] have recently presented an intricate EBV plasmid (pRTS-1; size including eGFP and luciferase cDNAs is 18.4 kb) that uses two fusion proteins, an optimized version of rtTA plus a tTS, to regulate expression from a dual tetracycline promoter. With pRTS-1 there was very high inducibility (e.g. by a factor of 140 000) for single clones in the luciferase assay, while other clones showed hardly any induction when eGFP was assayed. This suggests that for very high inducibility it may be

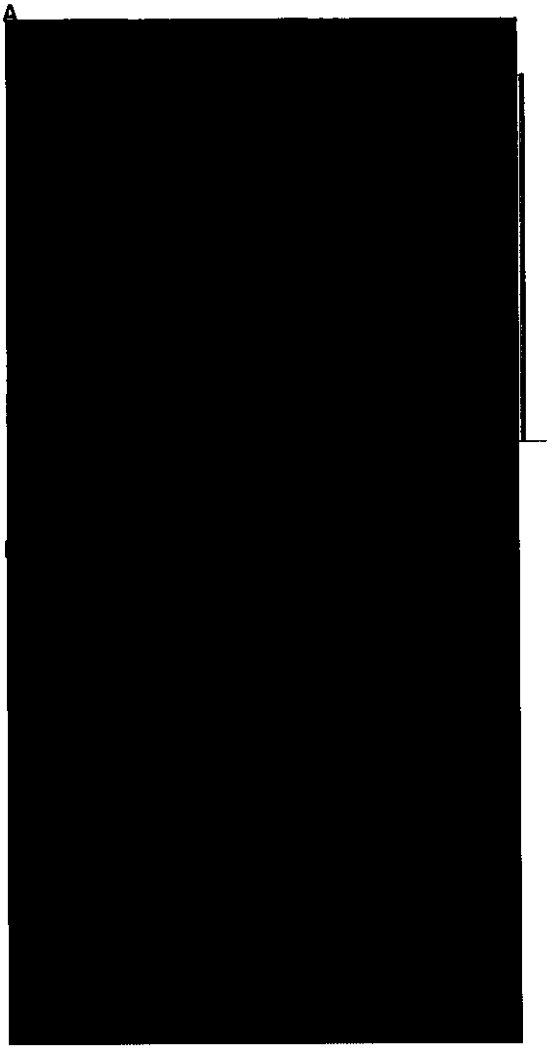

Fig. 3. Validation of pEBTet and pEBTetD vectors on the level of protein function. (A) Regulation of expression ETTh, which resides in the plasma membrane. Ergothioneine (ET) content was assayed by LC-MS/MS. The clearance equals initial rate of specific uptake (= uptake mediated by expressed carrier) divided by substrate concentration. For each of the bars, endogenous ET content was determined in parallel and subtracted from total ET content to yield the carrier-mediated increase of ET during the incubation with substrate (1 min, 10 $\mu mol \cdot L^{-1}$). With nontransfected cells, no ET was detected. (B) Regulation of expression of the human organic cation transporter type 1 (OCT1h), which also resides in the plasma membrane. Transporter expression in 293 cells was assayed by initial rates of uptake of radiolabelled 1-methyl-4-phenylpyridinium (MPP^+). Uptake was measured by liquid scintillation counting. Nonspecific uptake into nontransfected cells due to diffusion, endocytosis, or binding was subtracted from total uptake to yield the carrier-mediated uptake of MPP^+ (1 min, 0.1 $\mu mol \cdot L^{-1}$) as shown.

necessary to perform clonal selection also with EBV vectors. However, clonal selection eliminates the main benefit of EBV vectors, i.e. to save set-up time. Our emphasis was therefore on the analysis of cell pools. We do not assume that our system is superior in terms of inducibility of single clones; moreover, as with other systems, stability over culture time is probably limited [11]. However, our results for pEBTetD cell pools in continuous culture up to 10 weeks indicate useful factors of inducibility on the level of protein function. Clearly, in many situations, e.g. in RNA interference experiments, an activity ratio of 10 : 1 is sufficient. Importantly and in contrast to pRTS-1, our vector is based on the simple TetR; the use of chimeric transcription factor domains with the inherent risk of multiple effects on gene expression is avoided.

In summary, we have developed plasmid pEBTetD for full establishment of doxycycline-inducible protein expression in human cell lines by just a single transfection. For closely matching cellular backgrounds we use continuous expression of the original TetR instead of TetR-transactivator fusion proteins. As clonal isolation is unnecessary and because of efficient episomal propagation of the Epstein–Barr vector, our approach saves 2–10 weeks of time.

Experimental procedures

Plasmid constructs

All constructs were assembled by standard cloning methods and confirmed by DNA sequencing. The backbone of pEBTet stems from pCEP-Pu [23]. The *Tn10*-derived TetR open reading frame and the CMV-*tetO2-tetO2* promoter were taken from pcDNA6/TR and pcDNA5/FRT/TO (Invitrogen, Karlsruhe, Germany), respectively. The nucleotide sequence of plasmid pEBTet (11 486 bases) is available online. pEBTetD (11 200 b) corresponds to pEBTet except for the bovine growth hormone polyadenylation site deletion region: CTCGAG CGATCGCGGC CGCGGGG (original pEBTet sequence underlined). cDNAs were inserted into the polylinkers of pEBTet or pEBTetD. The cDNA sequence of ETTh [9] corresponds to GenBank entry Y09881. For pEBTet/ETTh, the 5′ interface between cDNA and pEBTet is **AAGCTT** GAATTCTGCAGAT TCGA gccacc ATGCGGGA (polylinker in bold, Kozak motif in lower case, cDNA underlined); the 3′ interface is ATTTCTAGA **TCCAGCAC**. For pEBTetD/ETTh, the 5′ interface is identical; the 3′ interface is ATTTCTAGA TCCAGCACAGTG **GCGGCCGCGG**. Our cDNA of OCT1h [24] corresponds to GenBank entry X98332 except for 2 bases (228C > T and 1294A > G). For pEBTet/OCT1h, the 5′ interface is **TCGGATCC** gccacc ATG CCCAC; the 3′ interface is CTCTGCAG **CTCGAGTC**. For pEBTetD/OCT1h, both interfaces are identical. Our cDNA of the *SLC22A16* gene corresponds to GenBank entry NM_033125.2 except for two bases (244T > C and

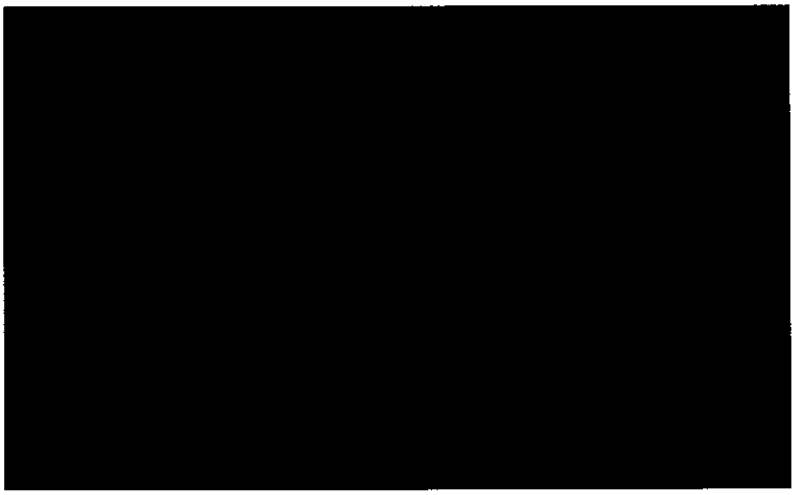

Fig. 4. Analysis of eGFP expression in 293 cells by flow cytometry. 293 cell pools stably transfected with either pEBTet/eGFP or pEBTetD/eGFP were cultured with or without 1 $\mu g \cdot mL^{-1}$ doxycycline for 20 h, resuspended, and then analyzed for eGFP fluorescence by flow cytometry. The fluorescence recorded for untransfected control cells corresponds to autofluorescence. Arithmetic means of fluorescence intensity were 3.4 (untransfected cells), 26 (pEBTetD/eGFP, off-state), 400 (pEBTetD/eGFP, on-state), 330 (pEBTet/eGFP, off-state), and 2700 (pEBTet/eGFP, on-state).

1293T > C) (D. Kropeit, R. Berkels & D. Gründemann unpublished results). For pEBTet/SLC22A16h, the 5′ interface is **GGTACC** CCCCCGGA; the 3′ interface is ATGCCTGC GGGGATCCAC TAGTAACGGC CGCC AGTGTG CTGGAATTCT GCAGATATCC ATCACAC **TGGCGGCC**. The cDNA of eGFP corresponds to GenBank entry U57609. For pEBTet/eGFP, the 5′ interface is **GGTACCG** CGGGCCCGGGATCCATC gccacc ATGG TGA; the 3′ interface is CAAGTAAA **GCGGCCGC**. For pEBTetD/eGFP, the 5′ interface is identical; the 3′ interface is CAAGTAAA **GCGGCCGCGG**.

Cell culture, transfection, and flow cytometry

293 cells (ATCC CRL-1573), a transformed cell line derived from human embryonic kidney, were grown at 37 °C in a humidified atmosphere (5% CO_2) in plastic culture flasks (Falcon 3112, Becton Dickinson, Heidelberg, Germany). The growth medium was Dulbecco's modified Eagle medium (Life Technologies 31885-023, Invitrogen) supplemented with 10% fetal bovine serum (PAA Laboratories, Cölbe, Germany). Medium was changed every 2–3 days and the culture was split every 5 days.

Cells were transfected with supercoiled plasmid DNA by lipofection with the Tfx-50 reagent according to the protocol of the vendor (Promega, Mannheim, Germany). From the next day on, stably transfected cells were selected with 3 $\mu g \cdot mL^{-1}$ puromycin (PAA Laboratories); puromycin was always present in subsequent cell culture to ascertain plasmid maintenance. To turn on protein expression, cells were cultivated for at least 20 h in regular growth medium supplemented with 1 $\mu g \cdot mL^{-1}$ doxycycline (195044, MP Biomedicals, Eschwege, Germany). For flow cytometry, cells were resuspended in growth medium and analysed on a FACSCalibur flow cytometer using CELLQUEST PRO software (BD Biosciences, San Jose, CA, USA).

Transport assays

For measurement of uptake of radiolabelled 1-methyl-4-phenylpyridinium (MPP^+), cells were grown in surface culture on 60 mm polystyrol dishes (Nunclon 150288, Nunc, Roskilde, Denmark) precoated with 0.1 $g \cdot L^{-1}$ poly L-ornithine in 0.15 M boric acid–NaOH, pH 8.4. Cells were used for uptake experiments at a confluence of at least 70%. Uptake was measured at 37 °C. After preincubation for at least 20 min in 4 mL of uptake buffer (in $mmol \cdot L^{-1}$: 125 NaCl, 25 Hepes–NaOH pH 7.4, 5.6 (+)glucose, 4.8 KCl, 1.2 KH_2PO_4, 1.2 $CaCl_2$, 1.2 $MgSO_4$), the buffer was replaced with 3 mL of $[^3H]MPP^+$ (at 0.1 $\mu mol \cdot L^{-1}$) in uptake buffer. Incubation was stopped after 1 min by rinsing the cells four times each with 4 mL ice-cold uptake buffer. Subsequently, the cells were solubilized with 0.1% v/v Triton X-100 in 5 $mmol \cdot L^{-1}$ Tris-HCl pH 7.4, and radioactivity was determined by liquid scintillation counting.

Uptake of ergothioneine (10 $\mu mol \cdot L^{-1}$) was determined by LC-ESI-MS/MS. Cells were assayed and washed as above, solubilized with 4 $mmol \cdot L^{-1}$ $HClO_4$ and stored at −20 °C. After centrifugation (1 min, 16 000 *g*, 20 °C) of the thawed lysates, 100 µL of the supernatant was mixed with 10 µL unlabelled MPP^+ iodide (5.0 $ng \cdot \mu L^{-1}$), which served as the internal standard. Of this mixture, 20 µL samples were analyzed by LC-MS/MS on a triple quadrupole mass spectrometer (TSQ Quantum, Thermo Electron, Dreieich, Germany). Atmospheric pressure ionization with positive electrospray was used. The LC system consisted of Surveyor LC-pump, autosampler, and Waters Atlantis HILIC silica column (length 100 mm, diameter 3 mm, particle size 5 µm). The solvent for isocratic chromatography (flow rate 250 $\mu L \cdot min^{-1}$) was made of methanol (70%) and 0.1% formic acid (30%). For quantification of ergothioneine by selected reaction monitoring, *m/z* 230 and

m/z 127 were selected as parent and fragment, respectively (collision energy: 24 V; scan time: 0.3 s). The area of the intensity versus time peak was integrated and divided by the area of the MPP^+ peak to yield the analyte response ratio. Linear calibration curves ($R^2 > 0.99$) were constructed from at least six standards which were prepared using control cell lysates as solvent. Sample analyte content was calculated from the analyte response ratio and the slope of the calibration curve, obtained by weighted linear regression.

For radiotracer assays, protein was measured by the bicinchoninic acid assay [25] with bovine serum albumin as standard. The protein content of MS samples was estimated from the response ratio for proline, which was calibrated against the bicinchoninic acid assay (4–6 matched cell dishes) for each MS session.

Northern blot analysis

Northern analysis was performed with ^{33}P-labelled double-stranded DNA probes essentially as described in [26]. Radioactivity was quantitated with a Fujifilm BAS-1800 II analyzer. Transcripts were normalized by reference to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels.

Calculations

Arithmetic means ($n = 3$) are given with SEM.

Drugs

Radiotracers used were as follows: MPP^+ iodide (H-3, 2.2 $kBq \cdot pmol^{-1}$, ART-150, ARC, St Louis, MO, USA).

Unlabeled compounds used were as follows: MPP^+ iodide (D-048, Sigma-Aldrich, Munich, Germany), L-(+)-ergothioneine (F-3455, Bachem, Bubendorf, Switzerland). All other chemicals were at least of analytical grade.

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life technologies™

CellLight® Mitochondria-RFP, BacMam 2.0

(Molecular Probes®)

Catalog Number	**C10601**
Size	**1 ml**
List Price	**(CAD) 465.00**

Description

CellLight® Mitochondria-RFP is a modified insect virus (baculovirus) expressing a fusion construct of a mitochondrial marker and red fluorescent protein. CellLight® reagents combine the utility and selectivity of fluorescent proteins with the transduction efficiency of BacMam technology, enabling unambiguous staining of organelles, cellular structures, and processes in live mammalian cells. They are provided in a ready-to-use format—simply add, incubate, and image—with highly efficient expression in cell lines, primary cells, stem cells, and neurons.

CellLight® Mitochondria-RFP is:

- **Highly Efficient:** >90% transduction of a wide range of mammalian cell lines, including primary cells, stem cells, and neurons
- **Fast and Convenient:** Simply add CellLight® Mitochondria-RFP reagent to your cells, incubate overnight, and image – or store frozen, assay-ready cells for later use
- **Safe:** Cell Light reagents are non-replicating in mammalian cells, lack of observable

cytopathic effect, and are suitable for biosafety level (BSL) 1 handling
- **Flexible:** You can co-transduce more than one BacMam reagent for multiplex experiments or co-localization studies and tightly control expression levels by just varying the dose

CellLight® Reagents Principle
CellLight® reagents are fusion constructs of signal peptides or cell structure proteins with premiere emGFP, TagRFP or CFP for accurate and specific targeting to sub-cellular compartments and structures. A variety of targets, including cytoskeleton, mitochondria, and secretory compartments, are available for convenient multiplexing, co-localization studies, and imaging of dynamic cellular processes where high spatial and temporal resolution is required. The CellLight® reagents tolerate fixation with formaldehyde and are therefore compatible with fixed-cell analysis.

BacMam Technology
The BacMam technology is based on an insect virus (baculovirus) for efficient transduction and transient expression in mammalian cells. The baculovirus has been modified to include an expression cassette containing the CellLight® fusion construct.

BacMam 2.0 incorporates elements that help greatly enhance transduction efficiency and expression levels: a pseudotyped capsid protein for more efficient cell entry, an enhanced CMV promoter, and the Woodchuck hepatitis post-transcriptional regulatory element (WPRE).

Baculoviruses do not replicate in mammalian cells and thus have an excellent safety profile and lack cytopathic effects on cells.

Work Flow Convenience
Unlike expression vectors, BacMam reagents enable titratable and reproducible transient protein expression. There is no need for harsh transfection methods or tedious cloning. To achieve highly efficient expression even in sensitive cells, such as stem cells, neurons, and primary cells, just add CellLight® reagents to cells in complete medium, incubate, and image the next day. Alternatively, mix CellLight® reagent and cells at the time of plating.

Co-transduction efficiencies are high allowing multiple CellLight® reagents to be readily used in the same experiment when multiple structures or pathways need to be labeled. CellLight® reagents also tolerate fixation with formaldehyde and are thus compatible with antibody-based fixed-cell analysis.

Typically, transiently transduced cells express fusion protein for about five days, though in slowly dividing cells, such as some primary cell types, expression has been demonstrated for up to two weeks; in terminally differentiated neurons we have recorded images more than three weeks after

transduction.

Related Products

We offer a range of BacMam-based reagents beyond CellLight® reagents, including the **BacMam GFP Transduction Control** that is ideal to test out the technology and optimize transduction conditions, Premo™ Biosensors, including **Premo™ Autophagy Sensor**, ion channel drug targets, pathway analysis kits, and more.

Learn more about these products and the BacMam technology

See other imaging tools and reagents

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Regulatory Statement:	For Research Use Only. Not for any animal or human therapeutic or diagnostic use.
Color:	Red-Orange, Orange
Quantity:	1 vial
Label or Dye:	RFP (TagRFP)
Emission Class:	Visible
Excitation Class:	Visible
Flow Cytometer Laser Lines:	532

CellLight Reagents *BacMam 2.0* Quick Reference

CellLight Reagents *BacMam 2.0*

Material Safety Data Sheet

Version 4.3
Revision Date 12/01/2011
Print Date 03/06/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : Cholera Toxin Vibrio cholerae

Product Number : C8052
Brand : Sigma
Product Use : For laboratory research purposes.

Supplier : Sigma-Aldrich Canada, Ltd
2149 Winston Park Drive
OAKVILLE ON L6H 6J8
CANADA

Manufacturer : Sigma-Aldrich Corporation
3050 Spruce St.
St. Louis, Missouri 63103
USA

Telephone : +1 9058299500
Fax : +1 9058299292
Emergency Phone # (For both supplier and manufacturer) : 1-800-424-9300
Preparation Information : Sigma-Aldrich Corporation
Product Safety - Americas Region
1-800-521-8956

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Bowel

WHMIS Classification

D2B Toxic Material Causing Other Toxic Effects — Moderate skin irritant
Moderate eye irritant

GHS Classification

Acute toxicity, Oral (Category 4)
Acute toxicity, Dermal (Category 4)
Skin irritation (Category 3)
Acute aquatic toxicity (Category 3)
Chronic aquatic toxicity (Category 3)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Warning

Hazard statement(s)

H302 + H312	Harmful if swallowed or in contact with skin.
H316	Causes mild skin irritation.
H412	Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P273	Avoid release to the environment.
P280	Wear protective gloves/ protective clothing.

HMIS Classification

Health hazard: 2
Chronic Health Hazard: *

Flammability: 0
Physical hazards: 0

Potential Health Effects

Inhalation May be harmful if inhaled. Causes respiratory tract irritation.
Skin Harmful if absorbed through skin. Causes skin irritation.
Eyes Causes eye irritation.
Ingestion Harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Cholera enterotoxin
Cholergen

CAS-No.	EC-No.	Index-No.	Concentration
Tris (hydroxymethyl) aminomethane			
77-86-1	201-064-4	-	>= 5.82 - <= 5.94 %
2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride			
1185-53-1	214-684-5	-	>= 31.3 - <= 31.9 %
Edetate disodium dihydrate			
6381-92-6	205-358-3	-	>= 1.83 - <= 1.87 %
Sodium chloride			
7647-14-5	231-598-3	-	>= 57.6 - <= 58.8 %
Exotoxin, vibrio cholerae			
9012-63-9	-	-	>= 0.5 - <= 2.5 %
Sodium azide			
26628-22-8	247-852-1	011-004-00-7	>= 0.96 - <= 0.98 %

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIREFIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas, Sodium oxides

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Avoid breathing dust.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator.For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection
Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Specific engineering controls
Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid
Colour	no data available

Safety data

pH	no data available
Melting point/freezing point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available
Density	no data available
Water solubility	no data available
Partition coefficient: n-octanol/water	no data available
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
Dimethyl sulfate, Acid chlorides, Halogenated hydrocarbon, Metals, Acids

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas, Sodium oxides
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
no data available

Inhalation LC50

no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
Eyes: no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Ingestion	Harmful if swallowed.
Skin	Harmful if absorbed through skin. Causes skin irritation.
Eyes	Causes eye irritation.

Signs and Symptoms of Exposure
Laboratory experiments in animals have shown sodium azide to produce a profound hypotensive effect, demyelination of myelinated nerve fibers in the central nervous system, testicular damage, blindness, attacks of rigidity, and hepatic and cerebral effects.

Synergistic effects
no data available

Additional Information
RTECS: Not available

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Harmful to aquatic life with long lasting effects.

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

WHMIS Classification

D2B	Toxic Material Causing Other Toxic Effects	Moderate skin irritant Moderate eye irritant

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

16. OTHER INFORMATION

Text of H-code(s) and R-phrase(s) mentioned in Section 3

Further information
Copyright 2011 Sigma-Aldrich Co. License granted to make unlimited paper copies for internal use only.
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Cholera toxin
Proposed Use Dose:	0.1 µg
Proposed Storage Dose:	1000 µg
LD_{50} (species):	250 µg

Calculation:

250 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 12500

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 1250

Comments/Recommendations:

Note from e-mail by Cindy Shao (03/29/2012) - 1mL medium is added to 100 ng toxin

Pathogen Regulation Directorate
Direction de la réglementation des agents pathogènes

100 chemin Colonnade Road, Loc.: 6201A
Ottawa, Ontario, Canada K1A 0K9

Tel: (613) 957-1779 **Fax: (613) 941-0596**

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

WHO Collaborating Centre for Biosafety

Centre collaborateur OMS pour les techniques de biosécurité

TO/À: Dale Laird
University of Western Ontario

DATE: FEBRUARY 2 0, 2012

FAX: 519-661-3420 **TEL:** 519-661-2111 ext:86827

PAGES TO FOLLOW / PAGES À SUIVRE: 1

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* COMMENTS - COMMENTAIRES *

Please find attached a copy of your **'Compliance Letter'** concerning the purchasing of biological material from a Canadian distributor. The original "Letter" is being sent to you through regular mail.

Be advised, however, that if these products contain matter of animal origin (such as bovine serum, etc.) or animal pathogens, you will need to contact the Canadian Food Inspection Agency (CFIA) at (613) 221-7068 for their consideration.

Thank you.

Vous trouverez sous pli une copie de votre **'Lettre de conformité'** concernant l'achat de matières biologiques chez un distributeur Canadien. La copie originale de votre "Lettre" vous parviendra par la poste.

Veuillez noter, cependant, que si ces produits contiennent des substances d'origine animale (par exemple du serum bovin) ou des pathogènes animals, vous devez contacter l'Agence canadienne d'inspection des aliments (ACIA) au (613) 221-7068 afin d'obtenir leur approbation.

Merci

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

Compliance Letter No. | N° de lettre de conformité: C-12-1146
Expiry Date | Date d'expiration: 2014-02-09

Compliance Letter

This letter serves to confirm that the Pathogen Regulation Directorate has reviewed a Containment Level 2 checklist based on the requirements identified in the Laboratory Biosafety Guidelines, 3rd Ed., 2004 for the facility identified below, and found the information submitted acceptable.

Lettre de Conformité

Par la présente, la Direction de la réglementation des agents pathogènes confirme qu'elle a examiné la liste de contrôle de niveau de confinement 2 en vertu des exigences des Lignes directrices en matière de biosécurité en laboratoire, 3e édition, 2004 pour l'installation identifiée ci-dessous et a déterminé que l'information fournie est acceptable.

HPTA Registration No. | N° d'enregistrement en vertu de la LAPHT:
R-06-000598

Entity / Facility | Organisation / Installation:
University of Western Ontario
Department of Anatomy & Cell Biology

Attention | À l'attention de:
Dale Laird

Address | Adresse:
Dental Science Building
1151 Richmond Street
London, ON
N6A 5C1

Laboratory Room No(s). | N° du/des pièce(s) du/des laboratoire(s):
00076A.

Type of work | Description du travail:
in vitro only | *in vitro* seulement

Should you have any questions regarding this letter, please do not hesitate to contact our office:
permit-permis@phac-aspc.gc.ca or 613-957-1779.

Pour toutes questions concernant cette lettre, n'hésitez pas à nous contacter : permit-permis@phac-aspc.gc.ca ou 613-957-1779.

FEBRUARY 2 0, 2012
Date

Geneviève Lacroix
Lead Biosafety Inspector |
Inspecteur / Inspectrice principal(e) en biosécurité

Canada

S-03.00

3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Cholera Toxin
from *Vibrio cholerae*

Catalog Number **C8052**
Storage Temperature 2–8 °C

CAS RN 9012-63-9
Synonyms: Cholera enterotoxin; Choleragen

Product Description
Cholera toxin is the virulent factor from *Vibrio cholerae* that leads to severe diarrhea followed by dehydration in humans.[1,2] Several bacterial toxins are ADP-ribosyl-transferases with protein substrates. Many of the substrates ADP-ribosylated by bacterial protein toxins are G-proteins, which are involved in signal transduction and ADP-ribosylation is one of the more significant post translational modifications of proteins. The ADP-ribosylation activity of cholera toxin activates adenylate cyclase, resulting in the production of cyclic AMP by adenylate cyclase, which causes many metabolic alterations.[1,2]

Cholera toxin belongs to the AB_5–subunit family of toxins.[1] The native hexameric protein has a molecular mass of ~85 kDa and contains two subunits. It consists of a single A subunit (~27.2 kDa), responsible for the ADP-ribosylation activity, and five B subunits (~11.6 kDa each), which are arranged as a pentameric ring with an apparent 5-fold symmetry and are associated with the cell surface receptor binding and subsequent internalization (transmembrane transport) of the enzymatic component.[3,4]

A single isoelectric variant of the cholera toxin has been isolated, which crystallizes readily and reproducibly.[5] Cholera toxin has an isoelectric point (pI) of 6.6. Chromatographic properties, however, suggest a cationic surface is exposed at pH 7.0, which apparently resides in B subunit.[6]

The entire hexameric complex is required for toxic behaviour. Choleragenoid, the intact pentamer of B subunits, interacts with a ganglioside G_{M1} membrane receptor, but cannot activate adenylyl cyclase; whereas, the A subunit alone does not enter the cell.[7]

Due to the effect on adenylate cyclase, cholera toxin and its purified A subunit are frequently used for the study of signal transduction mechanisms. In addition, cholera toxin acts as an adjuvant through the stimulation of B lymphocytes.

The cholera toxin B subunit alone is used for track tracing in neurological research, taking advantage of G_{M1} ganglioside binding and retrograde transport. Tissue culture cells treated with cholera toxin are not killed and tissues of animals do not become necrotic.

The B subunit is non-toxic to cells and possesses no intrinsic adenylate cyclase activity. The cholera toxin B subunit (CTB) attaches to cells by binding to ganglioside G_{M1}.[8] As a result, it has been shown to be a good label for microglial cells (due to the enrichment of ganglioside G_{M1} on their cell surface), but not for oligodendrocytes or astrocytes.[9] The B subunit has been reported to be an excellent tracer for the study of axonal transport using immunohistochemical methods. Recently it has been widely used as a marker of membrane lipid rafts, which are membrane microdomains enriched with cholestrol and sphingolipids. These lipid rafts have an important role in cell signaling and protein trafficking.[10]

This product is the active, native cholera toxin (composed of the A and the B subunits). It is a lyophilized powder containing ~5% protein (Lowry-TCA). When reconstituted with water to a final concentration of 1 mg cholera toxin per ml, the solution will contain 0.05 M Tris buffer salts, pH 7.5, 0.2 M NaCl, 3 mM NaN_3, and 1 mM sodium EDTA.

Purity: ~95% (SDS-PAGE)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices

Preparation Instructions
Cholera toxin is soluble in water at a concentration of 10 mg/ml. Swirl bottles gently during reconstitution. Avoid vigorous pipetting of solutions that may lead to foaming. Solutions can be filtered through a 0.2 µm filter.

Storage/Stability
The product was prepared and packaged using aseptic technique and sealed under vacuum. Store the lyophilized powder and reconstituted solutions at 2–8 °C.

The product, as supplied, is stable 3 years when stored properly.

Solutions are reported to be stable for 1 year when stored at 2-8 °C and will lose biological activity after prolonged exposure to pH below 6 or above 8.[6] DO NOT FREEZE.

References

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ESS,NDH,MAM 10/07-1

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