

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:	Gerald M. Kidder
DEPARTMENT:	Phys/Pharm (UWO) and Children's Health Res. Inst.
ADDRESS:	MSB-DSB, UWO and LHSC (5th floor VRL)
PHONE NUMBER:	83132 / 55427
EMERGENCY PHONE NUMBER(S):	519-474-4258 or 519-857-4317 or 519-472-5782
EMAIL:	gerald.kidder@schulich.uwo.ca

Location of experimental work to be carried out :

Building :	DSB	Room(s):	00066, 00076A
Building :	DSB (HSAF)	Room(s):	6016
Building :	VRL (level 1 mouse work only)	Room(s):	CHRI labs

***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: **NSERC, CIHR**

GRANT TITLE(S): **Structural Determinants of Connexin Specificity (NSERC)**
Roles of Gap Junctional Coupling in Oogenesis (CIHR)
Cx43 Mutations Linked to Human Disease (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
Kevin Barr	kevin.barr@schulich.uwo.ca	Mar. 2003
Dyane Li	dli258@uwo.ca	Feb. 2010
Carolina Gillio-Meina	carolina.gillio-meina@schulich.uwo.ca	Sept. 2004
Paul Dyce	paul.dyce@schulich.uwo.ca	Feb. 2010

Use of Biological Agents

Our research explores the roles of connexins (gap junction proteins) and intercellular gap junctional communication in development and reproduction. We use genetically altered mice as well as primary cell cultures derived from them. Occasionally, we will use established cell lines (e.g. HeLa cells or TM4 Leydig cells) in cases where larger amounts of material are needed for analytical purposes. Transgenic mouse lines (gene insertions, knockouts, knockins, etc.) are generated by the London Regional Transgenic and Gene Targeting Facility, or obtained from other institutions, or purchased from commercial suppliers such as The Jackson Laboratory or the European Mutant Mouse Archive. In addition, some of our work involves human cumulus cell samples obtained from The Fertility Clinic at the London Health Sciences Centre.

Genetically altered mice: None of the strains we study incorporate activated oncogenes into the genome or express toxins and therefore are maintained in standard mouse housing. SCID (severe combined immune deficient) mice, used as graft hosts, are always housed in barrier. Additional (CL2+) procedures are applied to the handling of mice into which cells have been grafted that carry a genomically-integrated retroviral vector (see below).

Primary mouse cell cultures: Cultures are established from fetal or adult organs and maintained under level 1 containment until infected with virus designed to effect overexpression of specific mutant or wildtype connexins or growth factors that modify connexin expression. Under level 2 containment, HEK-293 GPG packaging cells are transfected with the AP2 retroviral vector plasmid (see attached vector design) using Lipofectamine. The transfected 293 cells produce infective virus with the retrovector which is then used to infect the primary cells. These infected cells are cultured in vitro for 1-4 days under level 2 conditions during which time they may be used for gap junctional coupling measurements (dye transfer assays or electrical coupling measurements), fixed for imaging by confocal immunofluorescence microscopy, or lysed with detergent for gene expression assay by qRT-PCR or western blotting. Infected cells and culture materials that have contacted the virus are bleached (10% bleach for 20 min.) and then autoclaved with biohazardous waste. Medium removed from infected cells is vacuumed into a beaker containing enough bleach to maintain a 1:10 bleach to liquid ratio. Following medium removal, the vacuum lines are decontaminated by running a 10% bleach solution through them.

Tissue grafts: In some cases, cells harbouring an integrated retrovector are grafted into adult SCID mouse hosts to allow longer-term development of the cells in vivo. The retrovector expresses a wildtype or mutant cellular protein. After up to four weeks of graft development in the hosts, the hosts are euthanized and the grafts are removed and prepared for histological or gene expression analysis with any waste tissue being decontaminated with bleach as described above. The bedding is scraped from the empty mouse cages into a garbage bag within the fume hood. The bag and cages are then sprayed/dunked in bleach and sent to be incinerated/washed. The mouse carcasses are

bagged and the outside of the bags wiped with Clidox, then the bags are taken to the incinerator. Any paperwork and cage cards within the room are bagged for autoclaving.

Human tissue samples/cell cultures: Ovarian follicular cells (cumulus granulosa cells) are obtained from The Fertility Clinic at the London Health Sciences Centre. The cells are cultured for 1-4 days in a level 2 facility. During this time they are used for gap junctional coupling assays, assayed for gene expression by qRT-PCR, ELISA, and/or western blotting, or fixed for imaging by confocal immunofluorescence microscopy. The cells and culture materials are then decontaminated with bleach as described above prior to autoclaving.

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

The Kidder laboratory studies the factors that control the development of eggs, sperm, and the embryos resulting from their union. The focus is on the signals that pass from cell to cell, coordinating the complex events that must take place to ensure successful reproduction. This intercellular signaling is brought about in two fundamental ways: via channels, localized in structures known as gap junctions, which provide conduits for the direct passage of small molecules from cell to cell (gap junctional intercellular signaling), and via the release of proteins from cells that bind to receptors on other cells, triggering specific physiological responses (paracrine signaling). We study both of these types of intercellular signaling and the interactions between them using both mice and cultured cells. Our research can be broken down into three broad objectives:

1. Understanding the role that specific structural features of connexins- the proteins that form the subunits of gap junctions- play in spermatogenesis (the development of sperm cells). This work utilizes mice with genetic mutations affecting different parts of the connexin protein. We carefully analyze the effects of these different mutations on the development and function of the testes and on the production of sperm in order to better understand how the connexin's structure dictates its various functions in spermatogenesis.
2. Understanding the roles that connexins play in oogenesis (the development of egg cells) and how those roles interact with paracrine signaling. Again, this research involves both mice and cultured cells. We employ mice with genetic mutations that delete or alter the structure of particular connexin proteins. Using cell cultures derived from mouse ovaries, we modify the expression of connexins or paracrine signals to determine their roles in oocyte development.
3. Applying our knowledge of the roles of gap junctional intercellular signaling in oogenesis to improve the outcome of assisted reproductive technologies in the fertility clinic. Using human ovarian follicle cells obtained from patients in a local clinic, we are testing the possibility that the level of expression of a connexin in a single follicle can be used to predict whether the oocyte from that follicle will produce a healthy embryo after the oocyte is fertilized.

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
<i>E. coli DH5-alpha</i>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	1	Life Technologies	<input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>AP2 retrovector</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	0.1	D. Laird, UWO	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input checked="" type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 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: 2+ classification based on viral-infected cells being grafted into animals

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	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	patient samples	Not applicable
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	mice tissues	2010-023, 2010-231
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> 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	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	HeLa HEK-293	2 2	D. Laird (UWO) " "
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	NRK N2A TM3	1 1 1	D. Laird (UWO) D. Bai (UWO) ATCC
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> 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 type(s) indicate PHAC or CFIA containment level required 1 2 2+ 3

Additional Comments: _____

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		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (unpreserved)	The Fertility Clinic, LHSC	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or		Not Applicable		Not Applicable

Tissues (preserved)				
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Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

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

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

Organism	Plasmid(s) Transformed	Plasmid(s) Selected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
E. coli			no	no	transgene amplification

** 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
adenovirus	AP2	D. Laird, UWO	connexins, paracrine factors	production of infectious virus encoding normal or mutant cellular proteins

** 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
- ◆ 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: Although the adenovirus in which the retrovector is packaged is replication

**competent, the retrovector, once integrated into the genome of the infected cells,
is replication defective.**

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

Containment Level req'd ?

7.2 Name of animal species to be used **mice**

7.3 AUS protocol # **2010-231**

7.4 List the location(s) for the animal experimentation and housing. **Health Sciences Animal Facility**

7.5 Will any of the agents listed in section 4.0 be used in live animals
 NO YES, specify: **Primary cells with integrated retrovector are grafted into mice.**

7.6 Will the agent(s) be shed by the animal:
 YES NO, please justify: **Retrovector is designed to be replication-defective.**

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)
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD₅₀ (specify species) of the toxin or hormone

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

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

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

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? YES NO - If **NO**, please proceed to Section 11.0

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

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

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

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

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

11.0 Plants

- 11.1 Do you use plants? YES NO - If **NO**, please proceed to Section 12.0
- 11.2 If YES, please give the name of the species.
- 11.3 What is the origin of the plant?
- 11.4 What is the form of the plant (seed, seedling, plant, tree...)?
- 11.5 What is your intention? Grow and maintain a crop "One-time" use
- 11.6 Do you do any modifications to the plant? YES NO
If yes, please describe:
- 11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
- 11.8 Is the CFIA permit attached? YES NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

- 12.1 Will any of the above agents be imported? YES, country of origin NO
If **NO**, please proceed to Section 13.0
- 12.2 Has an Import Permit been obtained from HC for human pathogens? YES NO
- 12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO
- 12.4 Has the import permit been sent to OHS? YES, please provide permit # NO

13.0 Training Requirements for Personnel Named on Form

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

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

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

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

Enter Your Name G.M. Kidder **Date:** 1 Feb. 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: **00066 and 00076A DSB, April 2011**
 NO, please certify
 NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-0002**

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:
Thorough washing of skin; for needlestick, pinch to increase cleansing blood flow from wound. Wipe affected skin area and discard in biohazard waste. Report incident to supervisor.

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 G.M. Kidder Date: 1 Feb. 2012

15.4 Additional Comments: _____

16.0 Approvals

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

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

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Maire Ryan
Date: Feb. 22, 2012

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

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

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

Info on Cell Line(s)

Cell Biology

ATCC® Number: **CRL-1714™** [Order this Item](#) Price: **\$329.00**

Designations: **TM3**
 Depositors: JP Mather
Biosafety Level: 1
 Shipped: frozen
 Medium & Serum: [See Propagation](#)
 Growth Properties: adherent
 Organism: *Mus musculus*
 Morphology: epithelial

- Related Links ▶**
[NCBI Entrez Search](#)
[Make a Deposit](#)
[Frequently Asked Questions](#)
[Material Transfer Agreement](#)
[Technical Support](#)
[Related Cell Culture Products](#)

Source: **Organ:** testis
Disease: normal
Cell Type: Leydig cell;

Cellular Products: prostaglandin F2a
 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.

- BioProducts**
[Cell, microbial and molecular genomics products for the life sciences](#)

Receptors: luteinizing hormone (LH); epidermal growth factor (EGF); androgen receptor, positive; estrogen receptor, positive; progesterone receptor, positive

BioServices

Tumorigenic: No
 Age: 11 to 13 days
 Gender: male

- [Bio-materials management; basic repository to complex partnership-level services](#)

The TM3 cell line responds to LH with an increase in cAMP production, but does not respond to follicle stimulating hormone (FSH).

Comments: The maintenance of responsiveness to LH is dependent upon serum lot.

BioStandards

In the presence of LH, the cells are capable of metabolizing cholesterol.
 Tested and found negative for ectromelia virus (mousepox).

- [Biological Reference Material and Consensus Standards for the life science community](#)

Propagation: **ATCC complete growth medium:** A 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium with 2.5mM L-Glutamine, 0.5mM Sodium Pyruvate, 1.2 g/L Sodium Bicarbonate and 15 mM HEPES, 92.5%; horse serum, 5%; fetal bovine serum, 2.5%

Cell Biology

ATCC® Number: **CRL-1570™** [Order this Item](#) Price: **\$329.00**

Designations: **NRK-49F**

Depositors: JE DeLarco

Biosafety Level: 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Rattus norvegicus* deposited as *Rattus* sp.

Morphology: fibroblast

Source: **Organ:** kidney
Disease: normal

Permits/Forms: In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

Applications: transfection host

Receptors: epidermal growth factor (EGF); multiplication stimulating activity (MSA)

Comments: NRK-49F cells are contact inhibited and very sensitive to transformation by chemicals and viruses. Cultures should be subcultured before they become confluent. If allowed to become confluent, the cells will transform.

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%

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 is recommended

Medium Renewal: Twice per week

Subculturing: Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach.

Add fresh culture medium, aspirate and dispense into new culture flasks.

Preservation: culture medium 95%; DMSO, 5%

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Cell Biology

ATCC® Number:

CCL-131™

Order this Item

Price:

\$279.00

Designations:

Neuro-2a

Depositors:

RJ Klebe

Biosafety Level:

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Mus musculus

Morphology:



Organ: brain

Strain: A

Source:

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

Herpes simplex virus

Virus Susceptibility:

Vesicular stomatitis virus
Human poliovirus 1

Antigen Expression:

H-2, a haplotype; *Mus musculus*, expressed modal number = 95; range = 59 to 193.

Cytogenetic Analysis:

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

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Cell Biology

ATCC® Number:

CCL-2™

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Price:

\$279.00

Designations:

HeLa

Depositors:

WF Scherer

Biosafety Level:

2 [Cells contain human papilloma virus]

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Homo sapiens

epithelial

Morphology:



Organ: cervix

Source:

Disease: adenocarcinoma

Cell Type: epithelial

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.

Permits/Forms:

Applications:

transfection host
screening for Escherichia coli strains with invasive potential

Human adenovirus 3
Encephalomyocarditis virus

Virus Susceptibility:

Human poliovirus 1
Human poliovirus 2
Human poliovirus 3

Amelogenin: X
CSF1PO: 9,10
D13S317: 12,13.3
D16S539: 9,10

DNA Profile (STR):

D5S818: 11,12
D7S820: 8,12
THO1: 7
TPOX: 8,12
vWA: 16,18

Related Links ▶

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Cell Biology

ATCC® Number: **CRL-1573™** [Order this Item](#) Price: **\$279.00**

Designations: **293 [HEK-293]**
 Depositors: FL Graham
Biosafety Level: 2 [CELLS CONTAIN ADENOVIRUS]

Shipped: frozen
 Medium & Serum: [See Propagation](#)

Growth Properties: adherent
 Organism: *Homo sapiens*
 epithelial

Morphology:  PHOTO

Source: **Organ:** embryonic kidney
 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

DNA Profile (STR): Amelogenin: X
 CSF1PO: 11,12
 D13S317: 12,14
 D16S539: 9,13
 D5S818: 8,9
 D7S820: 11,12
 THO1: 7,9.3
 TPOX: 11
 vWA: 16,19

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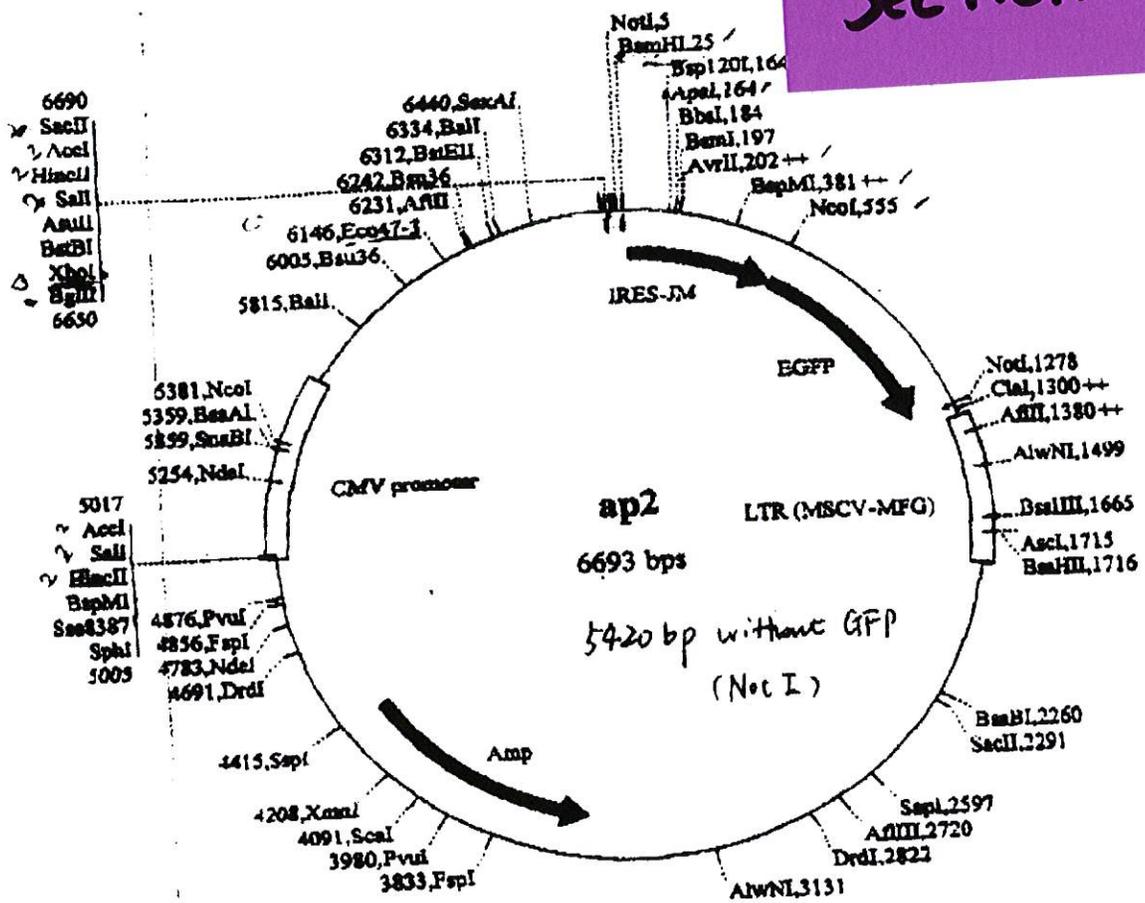
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