THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

BIO-UWO-0084

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous 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 biohazards being used.

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

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

PRINCIPAL INVESTIGATOR	Donglin Bai
SIGNATURE	
DEPARTMENT	Physiology and Pharmacology
ADDRESS	DSB00073
PHONE NUMBER	82569
EMERGENCY PHONE NUMBER(S)	
EMAIL	donglin.bai@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 00070

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR and NSERC
GRANT TITLE(S): The role of connexin43 in cardiac function (CIHR)
Cx43 mutations linked to human disease (CIHR)
Molecular domains determining gap junction channel properties (NSERC)

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

Names of all personnel working under Principal Investigators supervision in this location:

Xiang-Qun (Gregory) Gong
Li Xin
Andrew MacDonald

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

1.0 Microorganisms

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

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

_____
_____
_____

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

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E. coli DH5-alpha	☑ Yes O No	☑ Yes O No	☑ Yes O No	2 litre		O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

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

2.0 Cell Culture

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

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

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

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	✓Yes O No	HeLa, HEK	ATCC
Rodent	✓Yes O No	N2A, RIN, NRK, HL1	ATCC, Dr. Laird
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

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

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O 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/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
E. coli DH5-alpha	pcDNA3, pGFP RFP	Clontech	Cx43, Cx40, Cx50 Cx47, Cx26, Cx32	gap junction coupling

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
pLenti-Easy Adeno	pLenti-Easy pLP-Adeno	ABM Inc.	Cx43 and mutants	coupling level

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? ☑YES O NO

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

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ O NO

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

_____

_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

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

8.0 Biological Toxins

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

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

8.3 What is the LD_{50} (specify species) of the toxin mouse 8-10 ug/kg

8.4 How much of the toxin is handled at one time*? ~200 ml of 300 nM

8.5 How much of the toxin is stored*? 1 mg

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

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ☑ NO
If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O 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 plant? _____

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

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

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

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

10.8 Is the CFIA permit attached? O YES O NO
If NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

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

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

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

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

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE  _____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?

✓ YES, permit # if on-campus BIO-UWO-0084
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

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

SIGNATURE _____ Date: March 3, 2010

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

_____
_____
_____

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

_____
_____

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

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

Special Conditions of Approval:

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

Project description (Donglin Bai)

My research works are currently supported by 2 CIHR grants and a NSERC grant.
Here is a list of grant title, source and my role for each grant:
The role of connexin43 in cardiac function (CIHR, PI)
Cx43 mutations linked to human disease (CIHR, co-PI)
Molecular domains determining gap junction channel properties (NSERC, PI)

Our research goals are to understand how gap junction channels work and why mutations in gap junction proteins, connexins (Cxs), leading to human diseases. We have engineered or plan to engineer fluorescent protein tags, such as GFP and RFP, onto several Cxs (Cx43, Cx40, Cx50, Cx36, Cx26, Cx32, Cx47) to facilitate visual identification in live cells for Cx expression. We also engineered several disease-linked Cx mutants and artificial chimeras to reveal their function. These genetically engineered Cxs are expressed in gap junction deficient cell lines and in primary cultured cells to identify the functional alteration.

Here are some commonly used expression vector constructs are listed here with the URL-link for your information.

pcDNA3.1
https://www.lablife.org/g?a=vdb_view&old_id=24

pEGFP-N1
http://www.liv.ac.uk/physiology/ncs/catalogue/Cloning/pEGFP-N1.htm

RFP
http://www.lablife.org/p?a=vdb_view&id=g2%2eQL%5faGT2t1aghGKk1eSmYzQuUhkQ%2d

pLenti-Easy
http://www.abmgood.com/viralexp/LentiHis.php?csn=14&ssn=3884&dsn=4021

RFP not found.

pLenti-easy not found.

ATCC®
The Global Bioresource Center™

Search Catalog
Select a Category
Go

Login Search Options

About | Cultures and Products | Science | Standards | Deposit Services | Custom Services | Product Use Policy

ATCC Advanced Catalog Search » Product Details

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Print this Page

Cell Biology

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

Designations: HeLa

Depositors: WF Scherer

Biosafety Level: 2 [CELLS CONTAIN PAPOVAVIRUS]

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

PHOTO

Source:
Organ: cervix
Disease: adenocarcinoma
Cell Type: epithelial

Cellular Products:
keratin
Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]

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

Applications:
transfection host ([21491] Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)
screening for Escherichia coli strains with invasive potential [21447] [21491]

Virus Susceptibility:
Human adenovirus 3
Encephalomyocarditis virus
Human poliovirus 1
Human poliovirus 2
Human poliovirus 3

Reverse Transcript: negative

DNA Profile (STR):
Amelogenin: X
CSF1PO: 9,10
D13S317: 12,13.3
D16S539: 9,10
D5S818: 11,12
D7S820: 8,12
THO1: 7
TPOX: 8,12
vWA: 16,18

Related Links ▸
NCBI Entrez Search
Cell Micrograph
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

Cytogenetic Analysis: Modal number = 82; range = 70 to 164.
There is a small telocentric chromosome in 98% of the cells. 100% aneuploidy in 1385 cells examined. Four typical HeLa marker chromosomes have been reported in the literature.HeLa Marker Chromosomes: One copy of MI, one copy of M2, four-five copies of M3, and two copies of M4 as revealed by G-banding patterns. M1 is a rearranged long arm and centromere of chromosome 1 and the long arm of chromosome 3. M2 is a combination of short arm of chromosome 3 and long arm of chromosome 5. M3 is an isochromosome of the short arm of chromosome 5. M4 consists of the long arm of chromosome 11 and an arm of chromosome 19.

Isoenzymes: G6PD, A

Age: 31 years adult

Gender: female

Ethnicity: Black

HeLa Markers: Y

Comments: The cells are positive for keratin by immunoperoxidase staining.
HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences.
P53 expression was reported to be low, and normal levels of pRB (retinoblastoma suppressor) were found.

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

Subculturing: **Protocol:**

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

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

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

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
derivative:ATCC CCL-2.1
derivative:ATCC CCL-2.2
derivative:ATCC CCL-2.3

ATCC®
The Global Bioresource Center™

Search Catalog
Select a Category
Go

Login Search Options

About | Cultures and Products | Science | Standards | Deposit Services | Custom Services | Product Use Policy

ATCC Advanced Catalog Search » Product Details

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Print this Page

Cell Biology

ATCC® Number: CCL-131™ Order this Item
Price: $256.00

Designations: Neuro-2a
Depositors: RJ Klebe
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: *Mus musculus* (mouse)
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 (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

Virus Susceptibility:
Herpes simplex virus
Vesicular stomatitis virus
Human poliovirus 1

Reverse Transcript: negative

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

Related Links ▸
NCBI Entrez Search
Cell Micrograph
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

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 GTP binding to isolated protein, the turnover of microtubules in vivo, and the synthesis and assembly of microtubular protein [PubMed: 5263744]. The World Organization for Animal Health (OIE) uses the cells for routine diagnosis of rabies. (see: http://www.oie.int/Eng/Normes/Mmanual/A_00044.htm)Tested and found negative for ectromelia virus (mousepox).

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

Subculturing: **Protocol:**

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

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

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

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:
1023: Olmsted JB, et al. Isolation of microtubule protein from cultured mouse neuroblastoma cells. Proc. Natl. Acad. Sci. USA 65: 129-136, 1970. PubMed: 5263744
22161: Klebe RJ, Ruddle FH. Neuroblastoma: Cell culture analysis of a differentiating stem cell system. J. Cell Biol. 43: 69A, 1969.
29352: Naslavsky N, et al. Characterization of detergent-insoluble complexes containing the cellular prion protein and its scrapie isoform. J. Biol. Chem. 272: 6324-6331, 1997. PubMed: 9045652
29861: Kaneko K, et al. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. Proc. Natl. Acad. Sci. USA 94: 10069-10074, 1997. PubMed: 9294164
32459: Maestrini E, et al. A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor. Proc. Natl. Acad. Sci. USA 93: 674-678, 1996. PubMed: 8570614

Return to Top

Back to my Search

ATCC®
The Global Bioresource Center™

Search Catalog
Select a Category
Go

Login Search Options

About | Cultures and Products | Science | Standards | Deposit Services | Custom Services | Product Use Policy

ATCC Advanced Catalog Search » Product Details

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Print this Page

Cell Biology

ATCC® Number: CRL-6509™ Order this Item
Price: $355.00

Designations: NRK
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: Rattus norvegicus (rat)
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 (Nucleofection technology from Lonza
Roche FuGENE® 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
Preservation: **Freeze medium:** culture medium 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

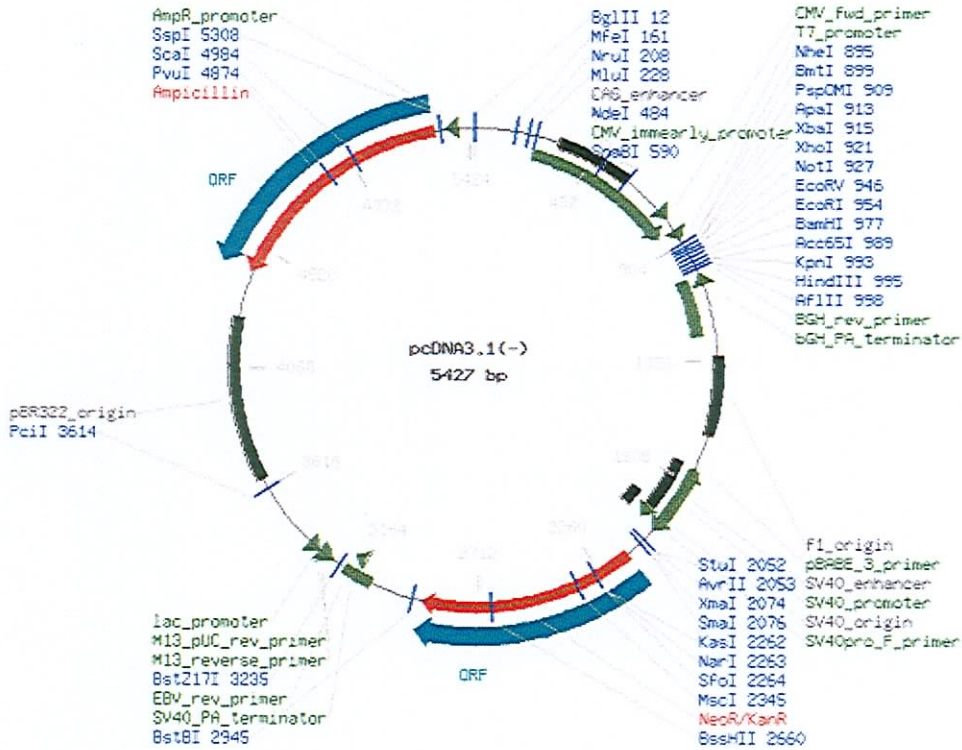
Related Links ▸
NCBI Entrez Search
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

Return to Top

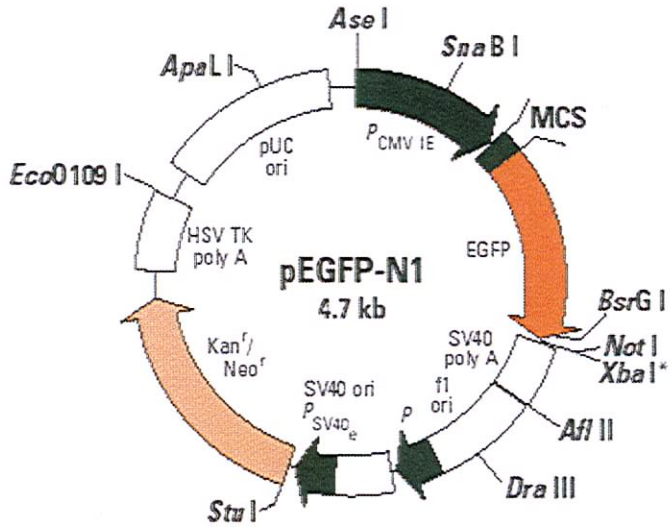
addgene **Vector Database**

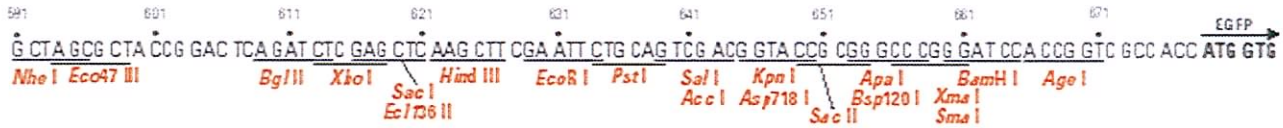
Vector Database is a list of plasmid backbones from publications and several companies, including cloning, mammalian expression, bacterial expression, and lentiviral and retroviral plasmids. The database is compiled by Addgene, and hosted on LabLife. LabLife does not sell or distribute any of the plasmids listed in this catalog.

Plasmid Name	pcDNA3.1(-)
Alt Names	pcDNA3.1-, pcDNA3.1
Source/Vendor	Invitrogen
Plasmid Type	Mammalian
Viral/Non-viral	Nonviral
Stable/Transient	Transient
Constitutive/Inducible	Constitutive
Promoter	CMV
Expression Level	High
Plasmid Size	5428
Sequencing Primer	T7 Fwd
Sequencing Primer Sequence	5'd[TAATACGACTCACTATAGGG]3'
Bacterial Resistance	Ampicillin
Mammalian Selection	G418, neo
Notes	Differs from other pcDNA3.1 in drug resistance; +/- refers to orientation of f1 ori.
Catalog Number	V79020, V79520
Plasmid Sequence	View Sequence



pEGFP-N1





Restriction Map and Multiple Cloning Site of pEGFP-N1. (Unique restriction sites are in color or bold.) The *Not* I site follows the EGFP stop codon. The *Xba* I site (*) is methylated in the DNA provided by CLONTECH. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*- host and make fresh DNA.

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

Sequence.

Restriction digest.

Excitation and emission Spectra

Description

pEGFP-N1 encodes a red-shifted variant of wild-type GFP (1-3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-N1 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-N1 is between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin-resistance cassette (neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex thymidine kinase gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette (P_{amp}) expresses kanamycin

resistance in *E. coli*. The pEGFP-N1 backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Vector	Size	Cat. #	GenBank Accession #
pEGFP-N1	20 µg	6085-1	U55762

Use

Fusions to the N-terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-N1 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-N1 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

Location of Features

- Human cytomegalovirus (CMV) immediate early promoter: 1-589
  Enhancer region: 59-465
  TATA box: 554-560
  Transcription start point: 583
  C->G mutation to remove *Sac* I site: 569
- MCS: 591-671
- Enhanced green fluorescent protein gene
  Kozak consensus translation initiation site: 672-682
  Start codon (ATG): 679-681; Stop codon: 1396-1398
  Insertion of Val at position 2: 682-684
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 871-876
  His-231 to Leu mutation (AÆT): 1373
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1552-1557 & 1581-1586
  mRNA 3' ends: 1590 & 1602
- f1 single-strand DNA origin: 1649-2104
  (packages the noncoding strand of EGFP)
- Bacterial promoter expression of Kan^r gene:
  -35 region: 2166-2171; -10 region: 2189-2194
  Transcription start point: 2201
- SV40 origin of replication: 2445-2580
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2278-2349 & 2350-2421
  21-bp repeats: 2425-2445, 2446-2466, & 2468-2488
  Early promoter element: 2501-2507
  Major transcription start points: 2497, 2535, 2541 & 2546
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2629-2631; stop codon: 3421-3423
  G->A mutation to remove *Pst* I site: 2811
  C->A (Arg to Ser) mutation to remove *Bss*H II site: 3157

- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3659-3664 & 3672-3677
- pUC plasmid replication origin: 4008-4651

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 745-724
- EGFP-C Sequencing Primer (#6478-1): 1332-1353

Propagation in *E. coli*

- Suitable host strains: DH5-alpha, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: 500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Prasher, D. C., *et al.* (1992) Primary structure of the *Aequorea victoria* green fluorescent protein. *Gene* **111**:229-233.
2. Chalfie, M., *et al.* (1994) Green fluorescent protein as a marker for gene expression. *Science* **263**:802-805.
3. Inouye, S. & Tsuji, F. I. (1994) *Aequorea* green fluorescent protein: Expression of the gene and fluorescent characteristics of the recombinant protein. *FEBS Letters* **341**:277-280.
4. Cormack, B., *et al.* (1996) FACS-optimized mutants of the green fluorescent protein (GFP). *Gene* **173**:33-38.
5. Haas, J., *et al.* (1996) Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr. Biol.* **6**:315-324.
6. Kozak, M. (1987) An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* **15**:8125-8148.
7. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*, Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143-190.