Modification Form for Permit BIO-LRCC-0006

Permit Holder: Eva Turley

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)

Jenny Ma

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	
Approved Primary and Established Cells	rodent (primary), mouse fibroblast cells, human (established), MDA-MB-231, MCF7, Melanoma WM1552C, Ovarian cancer SKOV3, OVCAR, rodent (established), Rhamm-/-MEF, CD44-/-MEF, Rhamm-/-	
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	plasmids: pCDNA3.1, Ph-Apr-1-Neo, pH-Apr-1-Hygro, pGEX-2TK, pPAL7	
Approved Use of Animals	mus musculus	
Approved Biological Toxin(s)		Botox, purified protein (Allergan). level 2
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: Nov 24, 2011

Date of Last Modification (if applicable): _____

BioSafety Officer(s)*: Mark Ryder MAR. 26/12

***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:** _____

Botox is used for many medical indications including spastic bladder, migraines, eye ticks and excessive sweating in the hands, feet and armpits. Currently, these medical indications require multiple injections of botox, often at up to 20-30 sites. For example, to treat spastic bladder syndrome, a catheter is inserted into the ureter and multiple injections are made on the inside of the bladder wall. These procedures are therefore often painful and require frequent injections (e.g. typically every 3 months). We have developed a hyaluronan-phospholipid topical formulation that both transports proteins across the skin and since this formulation inserts onto cell membranes, increases retention of proteins within the applied site. For example, we have delivered an 85kDa protein transdermally and also increased skin penetration and retention of vaccine peptides. Since this formulation increases retention of proteins by approximately 4 fold, it may offer advantages to injection in addition bypassing the need for delivery by injection. We propose to determine if labeled (FITC)-Botox is similarly transported across skin and retained in the muscle and dermal skin layers. We also propose to develop a formulation that could be applied by catheter to the inner wall of the bladder where botox would be released slowly, negating the need to injection and prolonging the time required for re-application.

We propose to initially apply botox formulated in hyaluronan-phospholipid topically to the back shoulders of mice that have been shaved. Injections of botox into the subcutaneous muscle layer of the skin will be used as a positive control. 5 units of botox will be applied or injected to each site. The areas of application/injection will be marked with a water insoluble pen and then animals will be euthanized at 24-96 hrs after application and the marked area will be sampled with a biopsy punch. The harvested skin will be snap frozen, cryostat tissue sections prepared and mounted then examined with a confocal microscope. The amount of fluorescence will be quantified with image analyses programs.

This project is in collaboration with Dr. Arjang Yazdani, who is licensed to purchase botox.

1. LD50: 0.1µg/kg (established in mice)=1MU
2. 0.1 MU
3. 10 MU
4. Yes, the biological toxin will be administered to BL6 female mice (6mos to 1 yr age). 0.001MU will be mixed with a cream and applied to the shaved back. As controls, the mice will also be injected with the same dosage of toxin.

E-mail from PI

PrBOTOX COSMETIC®

ALLERGAN®

Botulinum toxin type A for injection Ph. Eur. • *Clostridium botulinum* type A neurotoxin complex (900kD)

Sterile vacuum-dried concentrate powder for solution for injection

100 and 200 Allergan units per vial

NEUROMUSCULAR PARALYTIC AGENT

PART I: HEALTH PROFESSIONAL INFORMATION

SUMMARY PRODUCT INFORMATION

Route of Administration	Dosage Form / Strength	Clinically Relevant Nonmedicinal Ingredients
• Intramuscular	Sterile vacuum-dried concentrate; powder for solution for injection; 100 and 200 Allergan units per vial	Albumin (human) *For a complete listing see Dosage Forms, Composition and Packaging section.*

INDICATIONS AND CLINICAL USE

BOTOX COSMETIC® (Botulinum toxin type A for injection) is indicated:

- for the treatment of of upper facial rhytides, including forehead, lateral canthus and glabellar lines.

Geriatrics (> 65 years of age): Studies specifically designed to determine the dose in elderly patients have not been performed. Dosages for the elderly are as for other adults. Initial dosing should begin at the lowest recommended dose for the specific indication.

Pediatrics (< 18 years of age): Use of BOTOX COSMETIC® is not recommended in children.

CONTRAINDICATIONS

BOTOX® is contraindicated in:

- patients who are hypersensitive to botulinum toxin type A or to any ingredient in the formulation or component of the container. For a complete listing, see the Dosage Forms, Composition and Packaging section of the product monograph.
- the presence of infection at the proposed injection site(s).

WARNINGS AND PRECAUTIONS

Serious Warnings and Precautions

- The term "Allergan unit" upon which dosing is based, is a specific measurement of toxin activity that is unique to Allergan's formulation of botulinum toxin type A. Therefore, the "Allergan units" used to describe BOTOX COSMETIC® activity are different from those used to describe that of other botulinum toxin preparations and the units representing BOTOX COSMETIC® activity are not interchangeable with other products.
- BOTOX COSMETIC® should only be given by physicians with the appropriate qualifications and experience in the treatment and the use of required equipment.
- Follow the recommended dosage and frequency of administration for BOTOX COSMETIC® (**See WARNINGS AND PRECAUTIONS, General** and **DOSAGE AND ADMINISTRATION**).

General: Use BOTOX® and BOTOX COSMETIC® contain the same active ingredient in the same formulation. Therefore adverse events observed with the use of BOTOX® also have the potential to be associated with the use of BOTOX COSMETIC®.

Use BOTOX COSMETIC® only as directed.

Do not use dosage recommendations and potency Units applied to other botulinum toxin products when using BOTOX COSMETIC®.

The safe and effective use of BOTOX COSMETIC® (botulinum toxin type A for injection) depends upon proper storage of the product, selection of the correct dose, and proper reconstitution and administration techniques.

Caution should be used when BOTOX COSMETIC® is used in the presence of inflammation at the proposed injection site(s) or when excessive weakness or atrophy is present in the target muscle.

Muscle weakness remote to the site of injection and other serious adverse effects (e.g. dysphagia when injected into the neck region) have been very rarely reported in the cosmetic application.

Patients or caregivers should be advised to seek immediate medical care if swallowing, speech or respiratory disorders arise.

Patients with a history of underlying neurologic disorders, dysphagia and/or aspiration should be treated with extreme caution. The botulinum toxin product should be used under specialist supervision in these patients and should only be used if the benefit of treatment is considered to outweigh the risk.

Injection intervals of BOTOX COSMETIC® should be no more frequent than every two months. Indication specific dosage and administration recommendations should be followed. In treating adult patients, if combined with non-cosmetic indications, the maximum cumulative dose should generally not exceed 6 Units/kg, up to a maximum of 360 Units, in a 3 month interval.

One unit (U) of BOTOX COSMETIC® corresponds to the calculated median intraperitoneal lethal dose (LD_{50}) in mice. The method utilized for performing the assay is specific to Allergan's product, BOTOX COSMETIC®. Due to specific details of this assay such as the vehicle, dilution scheme and laboratory protocols for the various mouse LD_{50} assays, Units of biological activity of BOTOX COSMETIC® cannot be compared to nor converted into Units of any other botulinum toxin or any toxin assessed with any other specific assay method. Therefore, differences in species sensitivities to different botulinum neurotoxin serotypes precludes extrapolation of animal-dose activity relationships to human dose estimates. The specific activity of BOTOX COSMETIC® is approximately 20 units/nanogram of neurotoxin protein complex.

This product contains human serum albumin, a derivative of human blood. Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases. A theoretical risk for transmission of Creutzfeldt-Jakob disease (CJD) also is considered extremely remote. No cases of transmission of viral diseases or CJD have ever been identified for albumin.

Carcinogenesis and Mutagenesis: Studies in animals have not been performed to evaluate the carcinogenic potential of BOTOX COSMETIC®. BOTOX COSMETIC® was not mutagenic in *in vitro* and *in vivo* mutagenicity studies. (See TOXICOLOGY Section for more information.)

Cardiovascular: There have been rare reports following administration of botulinum toxin of adverse events involving the cardiovascular system, including arrhythmia and myocardial infarction, some with fatal outcomes. Some of these patients had risk factors including preexisting cardiovascular disease. The exact relationship of these events to BOTOX COSMETIC®/BOTOX® is unknown.

Immune: Formation of neutralizing antibodies to botulinum toxin type A may reduce the effectiveness of BOTOX COSMETIC® treatment by inactivating the biological activity of the toxin. The critical factors for neutralizing antibody formation have not been well characterized. The results from some studies suggest that BOTOX® injections at more frequent intervals or at higher doses may lead to greater incidence of antibody formation. When appropriate, the potential for antibody formation may be minimized by injecting with the lowest effective dose given at the longest feasible intervals between injections.

As with all biologic products, an anaphylactic reaction may occur. Necessary precautions should be taken and epinephrine should be available.

Serious and/or immediate hypersensitivity reactions such as anaphylaxis and serum sickness have been rarely reported, as well as other manifestations of hypersensitivity including urticaria, soft tissue edema, and dyspnea. Some of these reactions have been reported following the use of BOTOX COSMETIC® either alone or in conjunction with other products associated with similar reactions. If such a reaction occurs, further injection should be discontinued and appropriate medical therapy immediately instituted. One fatal case of anaphylaxis has been reported in which the patient died after being injected with BOTOX COSMETIC® diluted with 5 ml of 1% lidocaine. The causal role of BOTOX COSMETIC® , lidocaine, or both cannot be reliably determined.

Neurologic: Extreme caution should be exercised when administering BOTOX COSMETIC® to individuals with peripheral motor neuropathic diseases (e.g., amyotrophic lateral sclerosis, or motor neuropathy) or neuromuscular junction disorders (e.g., myasthenia gravis or Lambert-Eaton syndrome). Patients with neuromuscular junction disorders may be at increased risk of clinically significant systemic effects including severe dysphagia and respiratory compromise from typical doses of BOTOX COSMETIC®. There have been rare cases of administration of a botulinum toxin to patients with known or unrecognized neuromuscular junction disorders where the patients have shown extreme sensitivity to the systemic effects of typical clinical doses. In some of these cases, dysphagia has lasted several months and required placement of a gastric feeding tube. **When exposed to very high doses, patients with neurologic disorders, e.g., pediatric cerebral palsy or adult spasticity, may also be at increased risk of clinically significant systemic effects.**

Ophthalmologic: In order to reduce the complications of ptosis, avoid injection near the levator palpebrae superioris, particularly in patients with larger brow-depressor complexes. Medial corrugator injections should be placed at least 1 cm above the bony supraorbital ridge. To reduce the occurrence of diplopia, injections of the lateral canthal lines should be outside the bony orbit, not medial to the vertical line through the lateral canthus. To reduce the occurrence of lip ptosis, injections should be above the insertion of the zygomaticus muscles.

Skin: As is expected for any injection procedure, localized pain, inflammation, paresthesia, hypoaesthesia, tenderness, swelling/edema, erythema, localized infection, bleeding and/or bruising have been associated with the injection. Needle-related pain and/or anxiety have resulted in vasovagal responses, including transient symptomatic hypotension and syncope. Care should be taken when injecting near vulnerable anatomic structures.

Special Populations: ***Pregnant Women:*** There are no adequate and well-controlled studies of BOTOX COSMETIC® administration in pregnant women. Studies in animals have shown reproductive toxicity. The potential risk for humans is unknown. BOTOX COSMETIC® should not be used during pregnancy. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential risks, including abortion or fetal malformations, which have been observed in rabbits.

Nursing Women: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when BOTOX COSMETIC® is administered to a nursing woman.

Pediatrics (2-18 years of age): Use of BOTOX COSMETIC® is not recommended in children.

Geriatrics (> 65 years of age): Studies specifically designed to determine the dose in elderly patients have not been performed. Dosages for the elderly are as for other adults. Initial dosing should begin at the lowest recommended dose for the specific indication.

Monitoring and Laboratory Tests: There are no specific requirements for laboratory test monitoring when patients are treated with BOTOX COSMETIC®.

ADVERSE REACTIONS

Adverse Drug Reaction Overview: In general, adverse reactions occur within the first few days following injection and while generally transient may have duration of several months or, in rare cases, longer.

Local muscle weakness represents the expected pharmacological action of botulinum toxin in muscle tissue. However, weakness of adjacent muscles associated with local diffusion and/or injection technique has been reported. Muscle weakness remote to the site of injection and other serious adverse effects (e.g. dysphagia when injected into the neck region) have been very rarely reported in the cosmetic application.

As is expected for any injection procedure, localized pain, inflammation, paresthesia, hypoaesthesia, tenderness, swelling/edema, erythema, localized infection, bleeding and/or bruising have been associated with the injection. Needle-related pain and/or anxiety have resulted in vasovagal responses, including transient symptomatic hypotension and syncope.

Clinical Trial Adverse Drug Reactions: *Because clinical trials are conducted under very specific conditions the adverse reaction rates observed in the clinical trials may not reflect the rates observed in practice and should not be compared to the rates in the clinical trials of another drug. Adverse drug reaction information from clinical trials is useful for identifying drug-related adverse events and for approximating rates.*

Safety was evaluated in two multicenter, double-blind, placebo-controlled, parallel group studies of identical design for the treatment of glabellar lines (N=535; 405 in the BOTOX COSMETIC®-treated group and 130 in the placebo-treated group). The most frequently reported treatment-related adverse events were headache (9.4% in the BOTOX COSMETIC®-treated group and 15.4% in the placebo-treated group) and blepharoptosis (3.2% in the BOTOX COSMETIC®-treated group and 0% in the placebo-treated group). Blepharoptosis is consistent with the pharmacologic action of BOTOX COSMETIC®, and may be technique related.

Adverse events that were reported as treatment-related and were reported in 1-3% of BOTOX COSMETIC®-treated patients are listed in decreasing order of incidence: injection site pain/burning/stinging (2.5%), face pain (2.2%), erythema (1.7%), local muscle weakness (1.7%), injection site edema (1.5%), ecchymosis (1.0%), skin tightness (1.0%), paresthesia (1.0%) and nausea (1.0%). Most adverse events reported were of mild-to-moderate severity and all were transient. In a multicenter, open-label, repeat injection study, 318 patients who had participated in one of the two double-blind studies and who had glabellar line severity of at least mild severity at maximum frown received 2 additional treatments of BOTOX COSMETIC®. In this study, adverse events were comparable in type, incidence, severity, and causality to those reported in the two placebo-controlled, double-blind studies.

In clinical studies where BOTOX COSMETIC® was administered for the treatment of forehead or periorbital wrinkles, treatment-related adverse events have been consistent with those for glabellar lines. Adverse events that were reported as treatment-related after treatment of horizontal forehead lines with 16 U of BOTOX COSMETIC® include: headache (20%), bruising (10%), eyelid swelling (1.5%), and aching or itching forehead (10%). Injecting well above the brow reduces the risk of ptosis.

Treatment-related adverse events associated with treatment of periorbital wrinkles include mild bruising (4-25%) and headache (5.6%); these events occurred at a similar rate on the placebo-treated side. In addition, eyelid droop or shape change, and pain have been reported. Rare cases of diplopia and an asymmetric smile due to injection of zygomaticus major have been reported. These complications can be avoided with adherence to the recommended injection location (**see WARNINGS AND PRECAUTIONS AND DOSAGE AND ADMINISTRATION SECTIONS**).

Abnormal Hematologic and Clinical Chemistry Findings: No specific trends in abnormal hematologic or clinical chemistry findings have been reported.

Post-Market Adverse Drug Reactions: BOTOX® and BOTOX COSMETIC® contain the same active ingredient in the same formulation. Therefore, adverse events observed with the use of BOTOX COSMETIC® also have the potential to be associated with the use of BOTOX®.

Adverse events after treatment with botulinum toxin include rare spontaneous reports of death, sometimes associated with anaphylaxis, dysphagia, respiratory compromise, pneumonia, and/or other significant debility. There have also been rare reports of adverse events involving the cardiovascular system, including arrhythmia and myocardial infarction, some with fatal outcomes. Some of these patients had risk factors including pre-existing cardiovascular disease. New onset or recurrent seizures have also been reported, typically in patients who are predisposed to experiencing these events. The exact relationship of these events to the botulinum toxin injection has not been established.

The following other adverse events have been reported since the drug has been marketed: abdominal pain; diarrhea; vomiting; pyrexia; anorexia; vision blurred; visual disturbance, hypoacusis; tinnitus; vertigo; facial palsy, facial paresis; brachial plexopathy; radiculopathy; syncope; hypoaesthesia; malaise; myalgia; myasthenia gravis; paraesthesia; allergic reaction, skin rash (including erythema multiforme, urticaria and psoriasiform eruption); pruritus; hyperhidrosis; alopecia, including madarosis.

These reactions are reported voluntarily from a population of uncertain size. The exact relationship of these events to botulinum toxin is unknown.

DRUG INTERACTIONS

Overview: No specific interactions have been reported.

Drug-Drug Interactions

Table 2: Established or Potential Drug-Drug Interactions

Proper name of drug	Ref	Effect	Clinical comment
aminoglycoside antibiotics or spectinomycin, or other medicinal products that interfere with neuromuscular transmission (e.g. neuromuscular blocking agents, both depolarizing (succinylcholine) and non-depolarizing (tubocurarine derivatives),lincosamides, polymyxins, quinidine, magnesium sulfate, and anticholinesterases).	T	Theoretically, the effect of botulinum toxin type A may be potentiated	The effect of botulinum toxin may be potentiated by aminoglycoside antibiotics or spectinomycin, or other drugs that interfere with neuromuscular transmission (e.g. tubocurarine-type muscle relaxants). Caution should be exercised when BOTOX COSMETIC® is used with amino glycosides (e.g. streptomycin, tobramycin, neomycin, gentamycin, netilmicin, kanamycin, amikacin), spectinomycin, polymyxins, tetracyclines, lincomycin or any other drugs that interfere with neuromuscular transmission.
Different botulinum neurotoxin serotypes	T	Unknown	The effect of administering different botulinum neurotoxin serotypes at the same time or within several months of each other is unknown. Excessive weakness may be exacerbated by administration of another botulinum toxin prior to the resolution of the effects of a previously administered botulinum toxin.

Legend: T = Theoretical

Drug-Food Interactions: Interactions with food have not been established.

Drug-Herb Interactions: Interactions with herbal products have not been established.

Drug-Laboratory Interactions: Interactions with laboratory tests have not been established.

DOSAGE AND ADMINISTRATION

Dosing Considerations:

- **For Intramuscular Use Only.**
- BOTOX COSMETIC® (Botulinum toxin type A for injection) should only be given by physicians with the appropriate qualifications and experience in the treatment and the use of required equipment.
- The term "Allergan unit" upon which dosing is based, is a specific measurement of toxin activity that is unique to Allergan's formulation of botulinum toxin type A. Therefore, the "Allergan units" used to describe BOTOX COSMETIC® activity are different from those used to describe that of other botulinum toxin preparations and the units representing BOTOX COSMETIC® activity are not interchangeable with other products.
- The use of one vial for more than one patient is not recommended because the product and diluent do not contain a preservative.
- Treatment should be initiated at the lowest recommended dose. This dose can be gradually increased in subsequent treatments to the maximum recommended dose, if needed.
- Injection intervals of BOTOX COSMETIC® should be no more frequent than every two months. Indication specific dosage and administration recommendations should be followed. In treating adult patients, if combined with non-cosmetic indications, the maximum cumulative dose should generally not exceed 6 Units/kg, up to a maximum of 360 Units, in a 3 month interval.

Recommended Dose and Dosage Adjustment

BOTOX COSMETIC® is reconstituted only with 0.9% sterile non-preserved saline. Dilutions resulting in 4.0 U - 7.0 U per 0.1 mL are generally recommended.

Glabellar lines: 4 U should be administered intramuscularly using a 30 gauge needle in each of 5 sites, 2 in each corrugator muscle and 1 in the procerus muscle for a total dose of 20 U.

In order to reduce the complication of ptosis, injection near the levator palpebrae superioris should be avoided, particularly in patients with larger brow-depressor complexes. Medial corrugator injections should be placed at least 1 cm above the bony supra-orbital ridge.

Forehead lines: 2-6 U should be injected intramuscularly at each of 4 injection sites in the frontalis muscle, every 1-2 cm along either side of a deep forehead crease, 2-3 cm above the eyebrows, for a total dose of up to 24 U.

Lateral canthus lines: Generally, 2-6 U should be injected bilaterally at each of 1-3 injection sites at a 2-3 mm depth, lateral to the lateral orbital rim, where most lines are seen when a smile is forced. Injection should be at least 1 cm outside the bony orbit, not medial to the vertical line through the lateral canthus, and not close to the inferior margin of the zygoma.

An injection of BOTOX COSMETIC® is prepared by drawing into a sterile 1.0 mL tuberculin syringe an amount of the properly diluted toxin (see Dilution Table) slightly greater than the intended dose. Air bubbles in the syringe barrel are expelled and the syringe may be attached to the electromyographic injection needle, preferably a 1.5 inch, 27 gauge needle. Injection volume in excess of the intended dose is expelled through the needle into an appropriate waste container to assure patency of the needle and to confirm that there is no syringe-needle leakage. A new sterile needle and syringe should be used to enter the vial on each occasion for dilution or removal of BOTOX COSMETIC®.

Lack of Response: There are several potential explanations for a lack or diminished response to an individual treatment with BOTOX COSMETIC®. These may include inadequate dose selection, selection of inappropriate muscles for injection, muscles inaccessible to injection, underlying structural abnormalities such as muscle contractures or bone disorders, change in pattern of muscle involvement, patient perception of benefit compared with initial results, inappropriate storage or reconstitution, as well as neutralizing antibodies to botulinum toxin. A neutralizing antibody is defined as an antibody that inactivates the biological activity of the toxin. However, there have been patients who continued to respond to therapy and demonstrated presence of neutralizing antibodies; the proportion of patients which lose their response to botulinum toxin therapy and have demonstrable levels of neutralizing antibodies is small.

The critical factors for neutralizing antibody production are the frequency and dose of injection. To reduce the potential for neutralizing antibody formation, it is recommended that injection intervals of BOTOX COSMETIC® should be no more frequent than two months. More frequent injections should not be required, as BOTOX COSMETIC® treatment reduces the severity of the facial lines for up to 120 days.

A suggested course of action when patients do not respond to BOTOX COSMETIC® injections is:

1) wait the usual treatment interval;
2) consider reasons for lack of response listed above;
3) more than one treatment course should be considered before classification of a patient as a non-responder;
4) test patient serum for neutralizing antibody presence.

Missed Dose: Missed doses may be administered as soon as is practical.

Administration: An injection of BOTOX COSMETIC® is prepared by drawing into a sterile 1.0 mL tuberculin syringe an amount of the properly diluted toxin (see Dilution Table, below) slightly greater than the intended dose. Air bubbles in the syringe barrel are expelled and the syringe may be attached to the electromyographic injection needle, preferably a 1.5 inch, 27 gauge needle. Injection volume in excess of the intended dose is expelled through the needle into an appropriate waste container to assure patency of the needle and to confirm that there is no syringe-needle leakage. A new sterile needle and syringe should be used to enter the vial on each occasion for dilution or removal of BOTOX COSMETIC®.

Reconstitution:

Parenteral Products: To reconstitute vacuum-dried BOTOX COSMETIC®, use sterile normal saline without a preservative; 0.9% Sodium Chloride Injection is the only recommended diluent. Draw up the proper amount of diluent in the appropriate size syringe. Since BOTOX COSMETIC® is denatured by bubbling or similar violent agitation, inject the diluent into the vial gently. Discard the vial if a vacuum does not pull the diluent into the vial. Record the date and time of reconstitution on the space on the label. BOTOX COSMETIC® should be administered within twenty-four hours after reconstitution.

During this time period, reconstituted BOTOX COSMETIC® should be stored in a refrigerator (2° to 8° C). Reconstituted BOTOX COSMETIC® should be clear, colorless and free of particulate matter. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration and whenever the solution and the container permit.

Table 6: Dilution

Resulting dose:	Quantity of Diluent Added (0.9% Sodium Chloride for Injection)	
Units per 0.1 mL	100 U Vial	200 U Vial
7.5 U	1.3 mL	2.6 mL
5.0 U	2.0 mL	4.0 mL
2.5 U	4.0 mL	8.0 mL

OVERDOSAGE:

In the event of overdosage or injection error, additional information may be obtained by contacting Allergan, Inc. at (800) 433-8871.

Overdose of BOTOX COSMETIC® is a relative term and depends upon dose, site of injection, and underlying tissue properties. Signs and symptoms of overdose are not apparent immediately post-injection. Should accidental injection or oral ingestion occur, or overdose be suspected, the person should be medically monitored for up to several weeks for progressive signs or symptoms of muscular weakness distant from the site of injection that may include ptosis, diplopia, swallowing and speech disorders, generalized weakness or respiratory failure. These patients should be considered for further medical evaluation and appropriate medical therapy immediately instituted, which may include hospitalization.

ACTION AND CLINICAL PHARMACOLOGY

Mechanism of Action: BOTOX COSMETIC® (Botulinum toxin type A for injection) is a sterile, vacuum-dried form of purified botulinum neurotoxin type A complex, produced from a culture of the Hall strain of *Clostridium botulinum* grown in a medium containing N-Z amine, glucose and yeast extract. It is purified to a crystalline complex consisting of the neurotoxin, a non-toxic protein and four major hemagglutinin proteins.

BOTOX COSMETIC® blocks neuromuscular conduction by binding to receptor sites on motor nerve terminals, entering the nerve terminals, and inhibiting the release of acetylcholine. When injected intramuscularly at therapeutic doses, BOTOX COSMETIC® produces partial chemical denervation of the muscle resulting in localized muscle paralysis. When chemically denervated, the muscle may atrophy, axonal sprouting may occur, and extrajunctional acetylcholine receptors may develop. There is evidence that reinnervation of the muscle may occur, thus reversing muscle weakness produced by localized injection of BOTOX COSMETIC®.

One Allergan unit of BOTOX COSMETIC® corresponds to the calculated median intraperitoneal lethal dose (LD_{50}) in mice, performed in a mouse potency assay. This assay method is specific to Allergan's product, BOTOX COSMETIC®. Due to specific method details such as the vehicle, dilution scheme and laboratory protocols for the various mouse LD_{50} assays, units of biological activity of BOTOX COSMETIC® can not be compared to or converted into units of any other botulinum toxin activity. The specific activity of BOTOX COSMETIC® is approximately 20 units/nanogram of neurotoxin protein complex .

Pharmacodynamics: When injected into neck muscles, BOTOX COSMETIC® reduces both objective signs and subjective symptoms of cervical dystonia (spasmodic torticollis). These improvements include reduced angle of head turning, reduced shoulder elevation, decreased size and strength of hypertrophic muscles, and decreased pain. Based on the results of well-controlled studies, 40-58% of patients with cervical dystonia would be expected to have a significant improvement in their symptoms.

The paralytic effect on muscles injected with BOTOX COSMETIC® reduces the excessive, abnormal contractions of blepharospasm associated with dystonia.

When used for the treatment of strabismus, it has been postulated that the administration of BOTOX COSMETIC® affects muscle pairs by inducing an atrophic lengthening of the injected muscle and a corresponding shortening of the antagonist muscle.

Following injection of BOTOX COSMETIC® some distant muscles have shown increased electrophysiologic neuromuscular jitter. This effect is not associated with other types of electrophysiologic abnormalities, or with clinical signs of weakness or symptoms regarding either safety or efficacy.

In the treatment of pediatric cerebral palsy patients with dynamic equinus foot deformity due to spasticity, BOTOX COSMETIC® injections into the gastrocnemius produce an improvement in ankle position (reduction in equinus) and an improvement in gait pattern due to increased heel-to-floor contact.

In the treatment of hyperhidrosis of the axilla (N=320), BOTOX COSMETIC®-treated patients demonstrated a responder rate based on gravimetric assessment of 95% at week 1 and 82% at week 16. The mean percentage reduction in sweat production in the BOTOX COSMETIC®-treated patients ranged from 83% at week 1 to 69% at week 16. Treatment response has been reported to persist for 4 to 7 months (average of 5.2 months) in patients (N=12) treated with 50 U per axilla. Repeat injections should be administered when effects from previous injections subside.

When used for the treatment of focal spasticity BOTOX COSMETIC® injected into upper limb muscles reduces the objective signs and subjective symptoms of spasticity. Improvements include reduction of muscle tone, increase in range of motion, and in some patients reduction of spasticity-related disability.

Pharmacokinetics: It is believed that little systemic distribution of therapeutic doses of BOTOX COSMETIC® occurs. BOTOX COSMETIC® is not expected to be presented in the peripheral blood at measurable levels following IM or intradermal injection at the recommended doses. The recommended quantities of neurotoxin administered at each treatment session are not expected to result in systemic, overt distant clinical effects, i.e. muscle weakness, in patients without other neuromuscular dysfunction. However, clinical studies using single fiber electromyographic techniques have shown subtle electrophysiologic findings consistent with neuromuscular inhibition (i.e. "jitter") in muscles distant to the injection site, but these were unaccompanied by any clinical signs or symptoms of neuromuscular inhibition from the effects of botulinum toxin.

STORAGE AND STABILITY:

- Store the vacuum-dried product either in a refrigerator at 2° – 8°C, or in a freezer at or below –5°C.
- Administer BOTOX COSMETIC® within 24 hours after the vial is removed from the freezer and reconstituted.
- During these 24 hours, reconstituted BOTOX COSMETIC® should be stored in a refrigerator (2° to 8°C).
- Reconstituted BOTOX COSMETIC® should be clear, colorless and free of particulate matter.
- Do not freeze reconstituted BOTOX COSMETIC®.
- At the time of use, product acceptability should be confirmed relative to the expiration date indicated on the product vial and outer box.

SPECIAL HANDLING INSTRUCTIONS:

All vials, including expired vials, or equipment used with the drug should be disposed of carefully as is done with all medical waste.

DOSAGE FORMS, COMPOSITION AND PACKAGING:

BOTOX COSMETIC® is available in 100 and 200 unit (U) sterile vials of *Clostridium botulinum* toxin type A in a vacuum-dried form without a preservative. One Allergan unit (U) corresponds to the calculated median lethal dose (LD_{50}) in mice using reconstituted BOTOX COSMETIC® and injected intraperitoneally.

The quantities of the ingredients in each vial are listed below:

INGREDIENTS	100 Allergan U Vial	200 Allergan U Vial
Clostridium botulinum toxin type A neurotoxin complex (900kD)	100 U	200 U
Human Serum Albumin	0.5 mg	1.0 mg
Sodium Chloride	0.9 mg	1.8 mg

PART III: CONSUMER INFORMATION
BOTOX COSMETIC®
(Botulinum toxin type A for injection)

This leaflet is part III of a three-part "Product Monograph" published when BOTOX COSMETIC® was approved for sale in Canada and is designed specifically for Consumers. This leaflet is a summary and will not tell you everything about BOTOX COSMETIC®. Contact your doctor or pharmacist if you have any questions about the drug.

ABOUT THIS MEDICATION

What it is used for: BOTOX COSMETIC® is used to treat upper facial lines, including forehead, crow's feet, and frown lines.

When it should not be used: It should not be used if:

- you are allergic or sensitive to any of the ingredients
- you have an infection in the muscles where it would normally be injected.
- you have any muscle disorders in other parts of your body, including myasthenia gravis, Eaton Lambert Syndrome or amyotrophic lateral sclerosis.

What the medicinal ingredient is: Botulinum toxin type A for injection is a sterile, form of purified botulinum neurotoxin type A complex

What the important nonmedicinal ingredients are: Albumin (human) and sodium chloride.

WARNINGS AND PRECAUTIONS

BEFORE you receive BOTOX COSMETIC® talk to your doctor or pharmacist if:

- you have myasthenia gravis or Eaton Lambert Syndrome, amyotrophic lateral sclerosis or another muscle disorder.
- you are allergic or sensitive to BOTOX COSMETIC®.
- you have an infection at a proposed injection site.
- you are scheduled to have surgery using a general anaesthetic
- you are taking or are likely to take antibiotics, especially aminoglycoside antibiotics
- you are pregnant or become pregnant while taking this drug. Repeated doses of BOTOX COSMETIC® given to rabbits during pregnancy have caused abortion or fetal malformations.
- you are nursing. It is not known whether this drug is excreted in human milk, but many drugs are excreted in human milk.

BOTOX COSMETIC® is for intramuscular use only.

BOTOX COSMETIC® should only be given by a physician with the appropriate qualifications and experience in the treatment and the use of required equipment

Seek immediate medical attention if swallowing, speech or respiratory problems arise.

Tell your doctor if you experience any difficulties in swallowing food while on BOTOX COSMETIC®, as it could be related to the dosage. Difficulty in swallowing food, ranging from very mild to severe, can persist for 2-3 weeks after injection, or longer.

Tell your doctor if you are taking other medicines, including any you have bought at your pharmacy, supermarket or health food shop.

INTERACTIONS WITH THIS MEDICATION

The effect of BOTOX COSMETIC® may be increased by aminoglycoside antibiotics (e.g. streptomycin, tobramycin, neomycin, gentamicin, netilmicin, kanamycin, amikacin), spectinomycin, polymyxins, tetracyclines, lincomycin or any other drugs that interfere with neuromuscular transmission.

SIDE EFFECTS AND WHAT TO DO ABOUT THEM

General: Pain, tenderness and/or bruising at the site of injection. Malaise (generally feeling unwell), lasting up to six weeks after injection with BOTOX COSMETIC®. Weakness and rarely, changes in the way the heart beats, chest pain, skin rash and allergic reaction (symptoms: shortness of breath, wheezing or difficulty breathing; swelling of the face, lips, tongue or other parts of the body; rash, itching or hives on the skin).

The following events have been reported rarely (<0.1%) since BOTOX COSMETIC® has been marketed: skin rash, itching, allergic reaction (including anaphylaxis), dysphagia, respiratory compromise, seizures, and facial paralysis. There have also been rare reports after botulinum toxin treatment of adverse events involving the cardiovascular system, including arrhythmia and myocardial infarction, some with fatal outcomes. Some of these patients had risk factors, including cardiovascular disease.

This is not a complete list of side effects. For any unexpected effects while taking BOTOX COSMETIC®, contact your doctor or pharmacist.

REPORTING SUSPECTED SIDE EFFECTS

To monitor drug safety, Health Canada collects information on serious and unexpected effects of drugs. If you suspect you have had a serious or unexpected reaction to this drug you may notify Health Canada by: toll-free telephone: 866-234-2345; toll-free fax: 866-678-6789; By email: cdsmpt@hc-sc.gc.ca

By regular mail: National AR Centre, Marketed Health Products Safety and Effectiveness, Information Division, Marketed Health Products Directorate, Tunney's Pasture, AL 0701C, Ottawa ON K1A 0K9

NOTE: Before contacting Health Canada, you should contact your physician or pharmacist.

MORE INFORMATION

This document plus the full product monograph, prepared for health professionals can be found by contacting the sponsor, Allergan at: 1-877-255-3746

BGTD: http://www.hc-sc.gc.ca/dhp-mps/brgtherap/index-eng.php

This leaflet was prepared by Allergan

July 2010

ALLERGAN Inc.
Markham, Ontario,Canada, L6G 0B5

72299EC10B

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Botulinum Toxin Type A
Proposed Use Dose:	0.01 µg
Proposed Storage Dose:	1 µg
LD_{50} (species):	0.1 µg

Calculation:

0.1 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 5

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = ***0.5***

Comments/Recommendations:

THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University Of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

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

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

PRINCIPAL INVESTIGATOR	Dr. Eva Turley
DEPARTMENT	Oncology, Cancer Research lab, LRCP
ADDRESS	790 Commissioners Rd. E. Room A4-931
PHONE NUMBER	519-685-8500 Ex. 53677 Lab Ex.53280
EMERGENCY PHONE NUMBER(S)	519-685-8500 Ex. 53280
EMAIL	Eva.Turley@lhsc.on.ca

Location of experimental work to be carried out: Building(s) LRCP building A Room(s)_A4-931, A4928, A4-824

*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: CBCF, NSERC, MITACS (Octoderma)

GRANT TITLE(S):
1. RHAMM / HMMR in Breast and Prostate Cancer progression
2. The role of RHAMM in wound repair.
3. Unconventional export and trafficking of RHAMM

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

Name	UWO E-mail Address	Date of Biosafety Training
Jenny Ma	Jenny.Ma@lhsc.on.ca	Jun 23, 2011
Conny Toelg	Conny.Toelg@lhsc.on.ca	Jun 24, 2011
Natalia Akentieva	Natalia.Akentieve@lhsc.on.ca	Jun 23, 2011
Pat Telmer	Patrick.Telmer@lhsc.on.ca	Jun 24, 2011
Siddika Pardhan	spardha@uwo.ca	Jun 23, 2011

Sallie Elhayek	selhayek@uwo.ca	Jun 24,2011

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

1. We use *E.coli* strain K12 bacteria for propagation of protein expression plasmids and for producing recombinant RHAMM proteins. We use these proteins as reagents for producing polyclonal antibodies, as function blockers of cell invasion/migration and proliferation in culture and *in vivo*, for characterizing binding characteristics *in vitro* and for screening peptide mimetic/small chemical libraries to identify Rhamm function blocking reagents.
   All reagents for bacterial work are stored in labeled areas of the laboratory and cold room. Liquid bacterial cultures are sterilized with bleach overnight before disposing. All bacterial plates and contaminated pipettes are disposed of in Biohazardous waste containers in strict accordance with London Health Sciences hazardous waste policies.

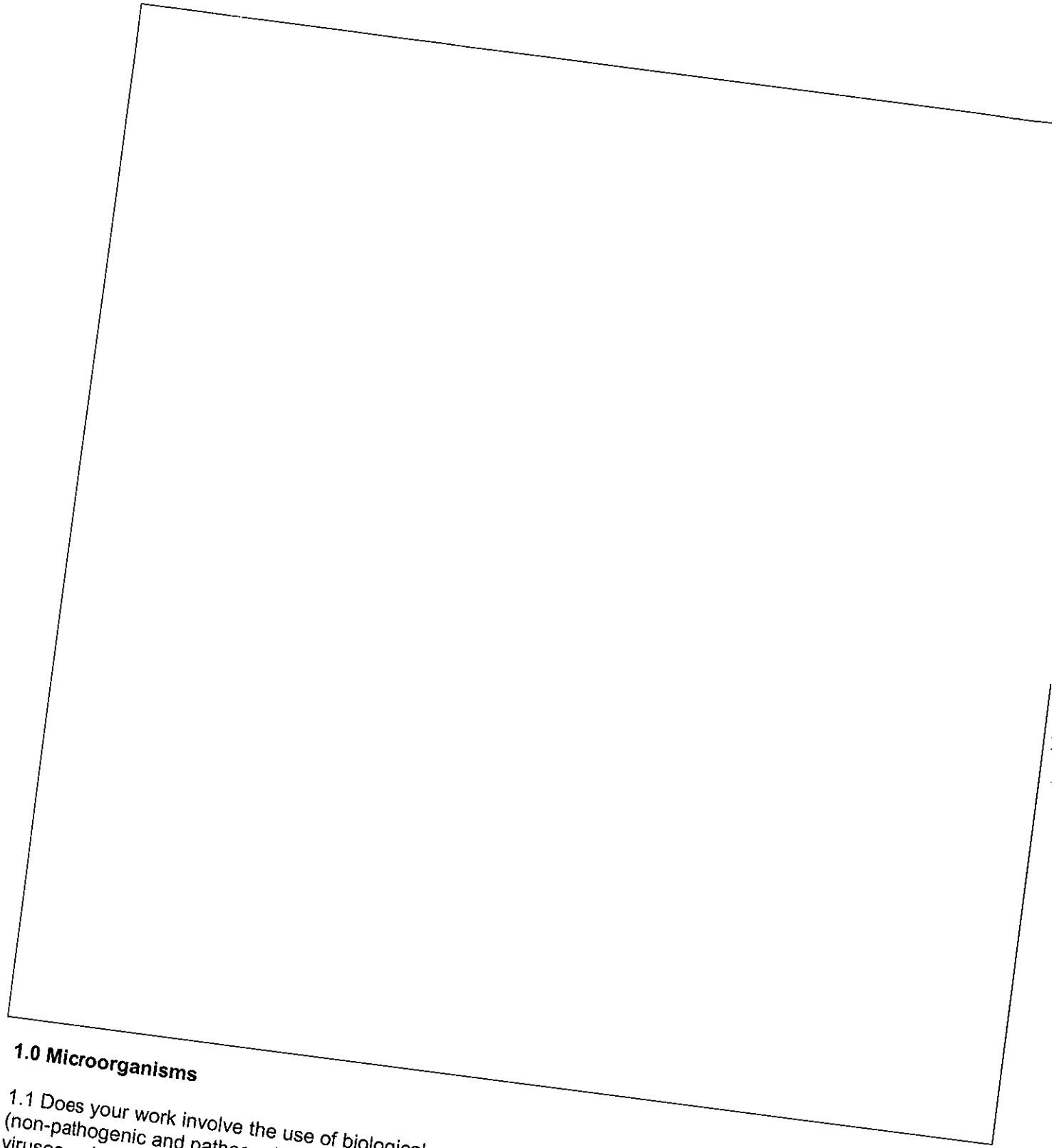
2. We use human cell lines including fibroblast, breast cancer, prostate cancer, and melanoma obtained from ATCC for in culture and *in vivo* (immune compromised mice) experiments.
   All cell culture reagents are disposed of Biohazardous waste containers in strict accordance with London Health Sciences hazardous waste policies. Liquid Media is decontaminated with bleach overnight before disposal. Frozen cell lines are kept in -80, -150 freezers or liquid nitrogen tank that are clearly labeled.

3. We have several varieties of rodent cells which we use to assess the function and localization of Rhamm and to characterize the effects of blocking Rhamm function on cell migration/proliferation. We use murine fibroblast lines obtained from ATCC along with primary cultures which are prepared by our lab. The primary cultures are muring embryonic fibroblasts, dermal fibroblasts, wound site fibroblasts, and bone marrow fibroblasts (stem cells). These are grown from explanted tissue and maintained in culture for approximately 8 passages. We obtain these primary cells from wildtype BL6 mice, Rhamm -/- BL6 mice, CD44-/- BL6 mice, and Rhamm-/-;CD44-/- BL6 mice.
   All cell culture reagents are disposed of Biohazardous waste containers in strict accordance with London Health Sciences hazardous waste policies. Liquid Media is decontaminated with bleach overnight before disposal.

New Info

Please include a one page research summary or teaching protocol.

My research program currently focuses upon defining the mechanisms by which the polysaccharide hyaluronan controls wound repair and the related process of tumour progression. Hyaluronan is a large negatively charged glycosaminoglycan produced by hyaluronan synthases. It functions both to organize the pericellular matrix and to activate signaling cascades that control cell migration and cell division. My laboratory identified, characterized and cloned the first cellular hyaluronan receptor, termed Rhamm (gene name HMMR). We are investigating the mechanisms by which this intracellular and extracellular protein controls wound repair, mesenchymal stem cell trafficking and breast/prostate cancer progression.



1.0 Microorganisms

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

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

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

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E.Coli	xO Yes O No	Ox Yes O No	Ox Yes O No	0.5L@0.70D frozen stock	Invitrogen	xO 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

Nov 18/11 per conversation with Jenny ma

*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? xO 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 x O No		Not applicable
Rodent	xO Yes O No	mouse fibroblastic cells	2009-060, 2009-051
Non-human primate	O Yes x O No		
Other (specify)	O Yes O No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	xO Yes O No	prostate stem cells, MDA-mb-231, MCF7, Melanoma WM1552C, Ovarian cancer SKOV3, OVCAR	2	Dr. Jim McCarthy's lab Minnesota, ATCC
Rodent	xO Yes O No	Rhamm-/-MEF, CD44-/-MEF, Rhamm-/-CD44-/MEF, 10T1/2, C3 10T1/2, MEF Rhamm+MEK, rat dermal fibroblasts, rat mesenchymal stem cells, Raw 264.7	2	Our mice, ATCC
Non-human primate	O Yes xO No			Level 1 per ATCC.org
Other (specify)	O Yes xO No			

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

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? xO YES O NO
If no, please proceed to Section 4.0

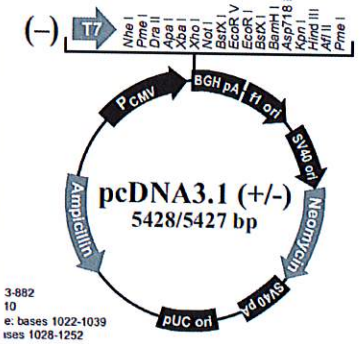
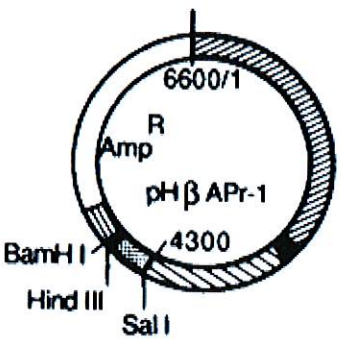
3.2 Indicate in the table below the Human Source Material to be used.

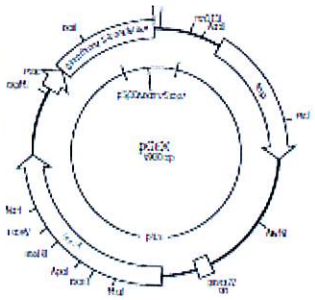
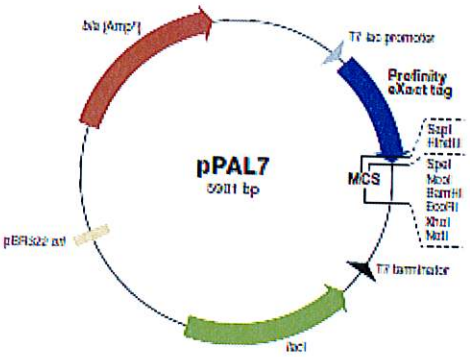
Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)	LRCP patients, Dr. Trevor Shepherd	O Yes xO Unknown		xO 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)	Isu Abxis Accumax tissue arrays	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? xO YES O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
E.coli K12 Strains: *DH5 alpha* *XL-1 Blue* *Bl21* *HB101* *TOP10* *All plasmids are propagated in one of these strains*	*pCDNA3.1*	*Parental Vector from INVITROGEN. Inserts were generated in laboratory or obtained from ATCC/NCBI* 	*containing the following inserts:* *-RHAMM cDNAs from mouse and human.* *-RHAMM fusion protein vector containing RHAMM fused to ZsGreen coding sequence.* *-Mutant – Active MEK1 coding sequence from mouse.* *-H-RAS from Mouse*	*-Alterations in cell motility* *-Transformation of fibroblasts* *-Fluorescent proteins for visualization of protein trafficking*
	pH-Apr-1-Neo *pH-Apr-1-Hygro* *REFERENCE:* **Gunning, P., J. Leavitt, G. Muscat, S. Y. Ng, and L. Kedes.** 1987. A human beta-actin expression vector system directs high-level accumulation of antisense transcripts. Proc. Natl. Acad. Sci. USA **84**:4831–4835.	*Plasmid map attached. Plasmid generated by colleague referenced. Neo denotes Neomycin selection cassette and Hygro indicates Hygromycin selection cassette* 	*-RHAMM cDNAs from mouse and Human*	*-Alterations in cell motility* *-Transformation of fibroblasts*

	pGEX-2TK	*GE-HEALTHCARE* 	*-RHAMM cDNAs from mouse and Human for recombinant protein production in E.coli strains listed*	*None. Protein production*
	pPAL7	*BIORAD*  Fig. 1. Profinity eXact pPAL7 vector.	*-RHAMM cDNAs from mouse for recombinant protein production in E.coli strains listed*	*None. Protein production*

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ xO NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ xO NO
- SV 40 Large T antigen O YES xO NO
- E1A oncogene O YES xO NO
- Known oncogenes xO YES, please specify: RHAMM/HMMR, RAS, MEK1 _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify_____ ~~O~~ NO

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES xO 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? xO YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used Mus Musculus (Mouse)

6.3 AUS protocol # _2009-060, 2009-051_____

6.4 Will any of the agents listed in section 4.0 be used in live animals xO YES, specify: Fibroblasts stably transfected with RHAMM cDNA will be used in Xenograft studies O NO

6.5 Will the agent(s) be shed by the animal: O YES xO NO, please justify: Cells used in study may form tumours in mammary fat pads, but these are not shed by the animal.

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES XO No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

7.3 If yes, please specify the animal(s) used:

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

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

_____

8.0 Biological Toxins

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

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

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

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

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

9.0 Insects

9.1 Do you use insects? O YES xO NO If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

_____
_____

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

10.0 Plants

10.1 Do you use plants? O YES xO NO If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

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

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

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

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

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

11.0 Import Requirements

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

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

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

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

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE 

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
xO YES, date of most recent biosafety inspection: Bio-LRCC-006 Level 2 Biosafety Inspection Dec. 10, 2010 Jane Ryder
O NO, please certify
O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): Bio-LRCC-0006

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.
No specific measures beyond standard biosafety associated with levels 1 and 2

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:
- Seek first aid or emergent intervention if required (refer to MSDS for Infectious Substances, or Blood-Borne Pathogen exposure protocol: http://intra.sjhc.london.on.ca/policy/search_res.php?polid=STF008&live=1
- Immediately report injury, exposure to supervisor
- Notify Occupational Health and Safety Services (OHSS) ASAP. Complete Workplace Occurrence Report: http://appserver.lhsc.on.ca/policy/search_res.php?polid=OHS011&live=1

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

SIGNATURE _____ Date: June 29 / 11

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: 24 Nov 2011

2) Safety Officer for the University of Western Ontario
SIGNATURE: J Stanley
Date: Nov 18 / 11

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Jane Ryder
Date: July 7, 2011

Approval Number: BIO-LRCC-0006 Expiry Date (3 years from Approval): Nov 23, 2014

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

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Info on cell Line(s)

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Cell Biology

ATCC® Number: HTB-26™ Order this Item

Price: $279.00

Designations: MDA-MB-231

Depositors: R Cailleau

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

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 (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

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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Isoenzymes:
AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 2
Me-2, 1-2
PGM1, 1-2
PGM3, 1

Age: 51 years adult

Gender: female

Ethnicity: Caucasian

Comments: The cells express the WNT7B oncogene [PubMed: 8168088].

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 100%
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 without CO2.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 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 30-2008
recommended serum:ATCC 30-2020
purified DNA:ATCC 45518
purified DNA:ATCC 45519
purified DNA:ATCC HTB-26D
purified RNA:ATCC HTB-26R

References:

1206: Brinkley BR, et al. Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. Cancer Res. 40: 3118-3129, 1980. PubMed: 7000337
22182: Cruciger Q, et al. Morphological, biochemical and chromosomal characterization of breast tumor lines from pleural effusions. In Vitro 12: 331, 1976.
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33021: Soker S, et al. Characterization of novel vascular endothelial growth factor (VEGF) receptors on tumor cells that bind VEGF165 via its exon 7-endoded domain. J. Biol. Chem. 271: 5761-5767, 1996. PubMed: 8621443

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Cell Biology

ATCC® Number: HTB-22™ Order this Item

Price: $279.00

Designations: MCF7

Depositors: CM McGrath

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

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 (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

Receptors: estrogen receptor, expressed

Antigen Expression: Blood Type O; Rh+

DNA Profile (STR):
Amelogenin: X
CSF1PO: 10
D13S317: 11
D16S539: 11,12
D5S818: 11,12
D7S820: 8,9
THO1: 6
TPOX: 9,12
vWA: 14,15

Cytogenetic Analysis: modal number = 82; range = 66 to 87.
The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.

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Isoenzymes:
AK-1, 1
ES-D, 1-2
G6PD, B
GLO-I, 1-2
PGM1, 1-2
PGM3, 1

Age: 69 years adult

Gender: female

Ethnicity: Caucasian

Comments: The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.The cells express the WNT7B oncogene [PubMed: 8168088].
Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha).
Secretion of IGFBP's can be modulated by treatment with anti-estrogens.

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: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10% .
Atmosphere: air, 95%; carbon dioxide (CO_2), 5%
Temperature: 37.0°C

Subculturing:
Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.
Note: if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures.

1. Remove culture medium to a centrifuge tube.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually 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. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:3 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

Doubling Time: 29 hrs

Related Products:
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
purified DNA:ATCC HTB-22D
purified RNA:ATCC HTB-22R
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:

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23217: Soule HD, et al. A human cell line from a pleural effusion derived from a breast carcinoma. J. Natl. Cancer Inst. 51: 1409-1416, 1973. PubMed: 4357757
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32582: Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc. Natl. Acad. Sci. USA 93: 136-140, 1996. PubMed: 8552591
32925: Zhu X, et al. Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc. Natl. Acad. Sci. USA 93: 6091-6095, 1996. PubMed: 8650224
38764: Bacus SS, et al. Differentiation of cultured human breast cancer cells (AU-565 and MCF-7) associated with loss of cell surface HER-2/neu antigen. Mol. Carcinog. 3: 350-362, 1990. PubMed: 1980588

Return to Top

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Cell Biology

ATCC® Number: HTB-77™ Order this Item

Price: $279.00

Designations: SK-OV-3 [SKOV-3]

Depositors: G Trempe, LJ Old

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source:
Organ: ovary
Disease: adenocarcinoma
Derived from metastatic site: ascites

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:** 1973

Applications: transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)

Tumorigenic: Yes

Antigen Expression: Blood Type B; Rh+

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11
D13S317: 8,11
D16S539: 12
D5S818: 11
D7S820: 13,14
THO1: 9,9.3
TPOX: 8,11
vWA: 17,18

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Cytogenetic Analysis: This is a hypodiploid human cell line. The modal chromosome number was 43, occurring in 63.3% of cells. The range was 42 to 45. The rate of higher ploidies was 32%. The del(1)(q21), der(13)t(1;?;13)(q11;?;q34), der(11)t(11;?) (q12), del(10)(q22) and 3 other marker chromosomes were common to most cells, and 3 others were found only in some cells. One N11 had the HSR segment from p11 to the distal end. The normal N10, N12, N15, N17 and N19 were absent. Others were either single or paired. There were from 1 to 6 rearranged and unassignable chromosomes. The X chromosome was either single or paired.

Isoenzymes:
AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 1-2
Me-2, 1
PGM1, 1-2
PGM3, 1

Age: 64 years

Gender: female

Ethnicity: Caucasian

Comments: SK-OV-3 cells are resistant to tumor necrosis factor and to several cytotoxic drugs including diphtheria toxin, cis-platinum and adriamycin.

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated McCoy's 5a Medium Modified, Catalog No. 30-2007. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO_2), 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.

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 30-2007
recommended serum:ATCC 30-2020

References:

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Cell Biology

ATCC® Number: HTB-161™ Order this Item

Price: $279.00

Designations: NIH:OVCAR-3

Depositors: R Ozols, TC Hamilton

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

PHOTO

Source:
Organ: ovary
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.

Isolation: **Isolation date:** 1982

Applications: transfection host (Roche FuGENE® Transfection Reagents)

Receptors: androgen receptor, positive; estrogen receptor, positive; progesterone receptor, positive

Tumorigenic: Yes

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11,12
D13S317: 12
D16S539: 12
D5S818: 11,12
D7S820: 10
THO1: 9,9.3
TPOX: 8
vWA: 17

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Cytogenetic Analysis: The cell line is aneuploid human female, with chromosome counts in the sub to near-triploid range. Several normal chromosomes (N11, N13, N14, N15, N16, N17, and N22) are clearly under-represented. Many of these missing chromosomes are represented in the large number of cytogenetically altered chromosomes identified as marker chromosomes. In addition to the marker chromosomes, there are a large number of other structurally abnormal and unassignable chromosomes that are not recognized as markers. Random loss and gain of chromosomes from cell to cell are noted in the exact chromosome counts and in the analysis of the karyotypes.

Isoenzymes:
AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 1
PGM1, 1
PGM3, 1

Age: 60 years

Gender: female

Ethnicity: Caucasian

Comments:
The NIH:OVCAR-3 line was established in 1982 by T.C. Hamilton, et al. from the malignant ascites of a patient with progressive adenocarcinoma of the ovary.
Forms colonies in soft agar and has an abnormal karyotype.
Resistant to clinically relevant concentrations of adriamycin, melphalan and cisplatin.
Both cultured cells and xenografts exhibit androgen and estrogen receptors.
Xenograft models have been used to show that treatment with 17 beta estradiol can induce progesterone receptors in this human ovarian carcinoma.
NIH:OVCAR-3 is an appropriate model system in which to study drug resistance in ovarian cancer, and the presence of hormone receptors should be useful for the evaluation of hormonal therapy.

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: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 20%.
Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO_2), 5%

Subculturing:
Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

- Remove and discard culture medium.
- Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
- 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.
- Add 2.0 to 3.0 ml of complete growth medium and aspirate cells by gently pipetting
- Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
- Incubate cultures at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended
Medium Renewal: Every 2 to 3 days

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

Related Products:
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001
recommended serum:ATCC 30-2020

References:

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Return to Top

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Cell Biology

ATCC® Number: CRL-1213™ Order this Item

Price: $429.00

Designations: FR (Rat Dermal Fibroblastic)

Depositors: B Smith

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: Rattus norvegicus (rat)

Morphology: fibroblast

Source:
Organ: skin
Strain: Sprague-Dawley
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.

Age: 18 days gestation

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%.
Temperature: 37.0°C

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Subculturing:

Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (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 10 to 20 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 37°C.
   Subcultivation Ratio: A subcultivation ratio of 1:3 is recommended
   Medium Renewal: 2 to 3 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 - 0.53mM EDTA in Hank's BSS (w/o Ca++, Mg ++): ATCC 30-2101
Phosphate-buffered saline: ATCC 30-2200
Cell culture tested DMSO: ATCC 4-X

Return to Top

Back to my Search

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Cell Biology

ATCC® Number: CCL-226™ Order this Item

Price: $279.00

Designations: C3H/10T1/2, Clone 8

Depositors: C Heidelberger

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: fibroblast

Source: **Strain:** C3H
Organ: embryo

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 FuGENE® Transfection Reagents)

Tumorigenic: No

Antigen Expression: H-2k

Cytogenetic Analysis: Mouse karyotype with a modal number of 80 chromosomes.

Age: embryo

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Comments:

C3H/10T1/2, Clone 8 was isolated by C. Reznikoff, D. Brankow and C. Heidelberger in 1972 from a line of C3H mouse embryo cells. [23019]
The cells are very sensitive to post confluence inhibition of cell division, do not produce tumors in syngeneic mice, have no background of spontaneous transformation, nor do they contain overt endogenous transforming murine leukemia or sarcoma viruses. [22697]
The cells are contact sensitive.
There is no detectable background spontaneous transformation.
They are highly susceptible to transformation by chemical agents. [1208]
Tested and found negative for ectromelia virus (mousepox).
NOTE: THE INOCULATION DENSITY, FEEDING AND HARVESTING SCHEDULES MUST BE FOLLOWED RIGIDLY IF THE LINE IS TO RETAIN ITS ESSENTIAL CHARACTERISTICS.
THE BATCH OF SERUM USED FOR GROWTH AND FOR TRANSFORMATION ASSAYS MAY AFFECT BOTH THE MORPHOLOGY OF THIS LINE AND THE RESULTS OBTAINED.
Monolayers established and maintained for the standard transformation assay should be free of all foci after 6 weeks. [1208]
The donor recommends that the line be used between the 5th and 15th passages only.

Propagation:

ATCC complete growth medium: The base medium for this cell line is Eagle's Basal medium with 2 mM L-glutamine , 1.5 g/L sodium bicarbonate and Earle's BSS. To make the complete growth medium, add the following components to the base medium: heat-inactivated fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO2), 5%

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. SUBCULTURE MUST BE DONE BEFORE THE CULTURE REACHES CONFLUENCE.
Subcultivation Ratio: Seed new flasks at 2000 viable cells/sq cm.
Medium Renewal: Once between subcultures if necessary

Preservation:

Freeze medium: Complete growth medium 95%; DMSO, 5%
Storage temperature: liquid notrogen vapor temperature

References:

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33039: Jain MK, et al. Molecular cloning and characterization of SmLIM, a developmentally regulated LIM protein preferentially expressed in aortic smooth muscle cells. J. Biol. Chem. 271: 10194-10199, 1996. PubMed: 8626582

Return to Top

Back to my Search

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Cell Biology

ATCC® Number: TIB-71™ Order this Item

Price: $279.00

Designations: RAW 264.7

Depositors: WC Raschke

Biosafety Level: 2

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: monocyte/macrophage

Source:
Tissue: ascites
Strain: BALB/c
Disease: Abelson murine leukemia virus-induced tumor
Cell Type: macrophage; Abelson murine leukemia virus transformed

Cellular Products: lysozyme [1207]

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: Biological response [92560]
transfection host (Roche FuGENE® Transfection Reagents)

Receptors: complement (C3) [1207]

Antigen Expression: H-2d

Age: adult

Gender: male

Comments: This line was established from a tumor induced by Abelson murine leukemia virus. They are negative for surface immunoglobulin (sIg-), Ia (Ia-) and Thy-1.2 (Thy-1.2)This line does not secrete detectable virus particles and is negative in the XC plaque formation assay. The cells will pinocytose neutral red and will phagocytose latex beads and zymosan. They are capable of antibody dependent lysis of sheep erythrocytes and tumor cell targets. LPS or PPD treatment for 2 days stimulates lysis of erythrocytes but not tumor cell targets.Data communicated in Feb. 2007 by Dr Janet W. Hartley, indicates the expression of infectious ecotropic MuLV closely related, if not identical, to the Moloney MuLV helper virus used in the original virus inoculum. The cells also express polytropic MuLV, unsurprisingly based on the mouse passage history of the virus stocks [PubMed 18177500].

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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: Subcultures are prepared by scraping.
For a 75 cm2 flask, remove all but 10 ml culture medium (adjust amount accordingly for other culture vessels). Dislodge cells from the flask substrate with a cell scraper; aspirate and add appropriate aliquots of the cell suspension into new culture vessels.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: Replace or add medium every 2 to 3 days.

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 30-2002
recommended serum:ATCC 30-2020

References:

1135: Ralph P, Nakoinz I. Antibody-dependent killing of erythrocyte and tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. J. Immunol. 119: 950-954, 1977. PubMed: 894031
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33076: Lokuta MA, et al. Mechanisms of murine RANTES chemokine gene induction by newcatle disease virus. J. Biol. Chem. 271: 13731-13738, 1996. PubMed: 8662857
33162: Taylor MF, et al. In vitro efficacy of morpholino-modified antisense oligomers directed against tumor necrosis factor-alpha mRNA. J. Biol. Chem. 271: 17445-17452, 1996. PubMed: 8663413
92560: Standard Practice for Testing for Biological Responses to Particles in Vitro. West Conshohocken, PA:ASTM International;ASTM Standard Test Method F 1903-98R03.
16173094: Hartley JW, et al. Expression of infectious murine leukemia viruses by RAW264.7 cells, a potential complication for studies with a widely used mouse macrophage cell line. Retrovirology. 4: 5:1, 2008. PubMed 18177500.

Return to Top

Back to my Search

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Cell Biology

ATCC® Number: CRL-2192™ Order this Item

Price: $279.00

Designations: NR8383 [AgC11x3A, NR8383.1]

Depositors: RJ Helmke

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: mixed, adherent and suspension

Organism: Rattus norvegicus (rat)

Morphology: macrophage

Source:
Strain: Sprague-Dawley
Organ: lung
Disease: normal
Cell Type: macrophage (alveolar);

Cellular Products: transforming growth factor beta (TGF beta); interleukin 1 (IL-1); interleukin 6 (IL-6)

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 3, 1983

Receptors: Fc

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Comments:

NR8383 (normal rat, August 3, 1983) was established from normal rat alveolar macrophage cells obtained by lung lavage.
The cells were cultured in the presence of gerbil lung cell conditioned medium for approximately 8 to 9 months.
Subsequently the requirement for exogenous growth factors was lost.
NR8383 cells were cloned and subcloned from single cells by limiting dilution, and then subcloned from soft agar three times.
The cells exhibit characteristics of macrophage cells:
Phagocytosis of zymosan and Pseudomonas aeruginosa, nonspecific esterase activity, Fc receptors, oxidative burst,IL-1, TNF beta and IL-6 secretion, and replicative response to exogenous growth factors.
The cells respond to appropriate microbial, particulate or soluble stimuli with phagocytosis and killing.
NR8383 cells respond to bleomycin by secreting latent transforming growth factor (TGF beta).
Stimulation with bleomycin also increases TGF beta mRNA expression.
These cells are sensitive to endotoxin.
LPS levels of 1 to 10 ng/ml inhibit replication by 50%.
LPS inhibition is nontoxic and reversible even after levels up to 0.001 mg/ml for extended periods.
The NR8383 cell line provides a homogenous source of highly responsive alveolar macarophages which can be used in vitro to study macrophage related activities.

Propagation:

ATCC complete growth medium: Ham's F12K medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 85%; heat inactivated fetal bovine serum, 15%
Temperature: 37.0°C

Subculturing:

Protocol: Cultures can be maintained by transferring floating cells to additional flasks. Adherent cells may be harvested by scraping. Upon reseeding, about one half of the cells will re-attach. Cultures are most successful when set up at a floating cell concentration of 1 to 4 X 10 exp5 viable cells/ml.
Medium Renewal: Two to three times weekly

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 30-2004
purified RNA:ATCC CRL-2192R

References:

22160: Hidalgo HA, et al. Pneumocystis carinii induces an oxidative burst in alveolar macrophages. Infect. Immun. 60: 1-7, 1992. PubMed: 1729174
22316: Helmke RJ, et al. A continuous alveolar macrophage cell line: comparisons with freshly derived alveolar macrophages. In Vitro Cell. Dev. Biol. 25: 44-48, 1989. PubMed: 2914814
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23190: Krieg DP, et al. Resistance of mucoid Pseudomonas aeruginosa to nonopsonic phagocytosis by alveolar macrophages in vitro. Infect. Immun. 56: 3173-3169, 1988. PubMed: 3141284
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23484: Griscavage JM, et al. Inducible nitric oxide synthase from a rat alveolar macrophage cell line is inhibited by nitric oxide. J. Immunol. 151: 6329-6337, 1993. PubMed: 7504017
23566: Henderson SA, et al. Nitric oxide reduces early growth response-1 gene expression in rat lung macrophages treated with interferon-gamma and lipopolysaccharide. J. Biol. Chem. 269: 25239-25242, 1994. PubMed: 7523382
36466: Huang S, et al. Rat KC cDNA cloning and mRNA expression in lung macrophages and fibroblasts. Biochem. Biophys. Res. Commun. 184: 922-929, 1992. PubMed: 1374243

Return to Top

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Cell Biology

ATCC® Number: CRL-2808™ Order this Item

Price: $338.00

Designations: WM1552C [Part of the Wistar Special Collection]

Depositors: M Herlyn

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: Spindle-shaped

PHOTO

Source:
Organ: skin
Tumor Stage: stage 3?
Disease: primary superficial spreading melanoma (SSM) (radial growth phase (RGP)/vertical growth phase (VGP))

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: Distribution of this cell line is governed by the Wistar Special Collection Material Transfer Agreement.

Isolation: **Isolation date:** February 8, 1988

Tumorigenic: Yes

Age: 72 years

Gender: male

Comments: The WM1552C line was established from a primary superficial spreading melanoma (SSM) in radial growth phase (RGP)/vertical growth phase (VGP) from the buttocks of a patient on 02/08/88.

Propagation:
ATCC complete growth medium: 2% Tumor Medium (Tu2%) containing a 4:1 mixture of MCDB 153 medium with 1.5 g/L sodium bicarbonate and Leibovitz's L-15 medium with 2 mM L-glutamine supplemented with 0.005 mg/ml bovine insulin, 1.68 mM CaCl2, and 2% fetal bovine serum.
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 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.
   An inoculum of 5 X 10(3) to 7 X 10(3) viable cells/cm2 is recommended. .
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 1 X 10(4) and 7 X 10(4) cells/cm2
Subcultivation Ratio: A subcultivation of 1:3 to 1:4 is recommended
Medium Renewal: Two to three times weekly

Preservation:

Freeze medium: Complete growth medium supplemented with an additional 8% fetal bovine serum and 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Doubling Time:

42 hours

Related Products:

recommended serum:ATCC 30-2020

References:

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89403: Satyamoorthy K, et al. Melanoma cell lines from different stages of progression and their biological and molecular analyses. Melanoma Res. 7: S35-S42, 1997. PubMed: 9578415

Return to Top

Back to my Search