The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: August 12, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Dr. Gideon Koren**
DEPARTMENT:	**Department of Physiology and Pharmacology**
ADDRESS:	**MBL- C111**
PHONE NUMBER:	**519-661-3128**
EMERGENCY PHONE NUMBER(S):	
EMAIL:	**gkoren@uwo.ca**

Location of experimental work to be carried out :

Building : **Robarts Research Institute** Room(s): **2220, 2226**

Building : _____ Room(s): _____

Building : _____ Room(s): _____

***For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **CIHR, POGO**

GRANT TITLE(S): **Hair Cortisol as a biomarker, Ifosfamide-induced nephrotoxicity**

UNDERGRADUATE COURSE NAME(IF APPLICABLE): _____

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

Name	UWO E-mail Address	Date of Biosafety Training
Lauren Hanly	**lhanly@uwo.ca**	**1-Oct-2008**
Evan Russell	**erussel5@uwo.ca**	**22-Jan-2009**

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

HK-2 cells are cultured and treated with the chemotherapy agent Ifosfamide, as well as several antioxidants in order to assess the nephrotoxic effects of Ifosfamide and the ability of antioxidants to attenuate it. These cells are stored and cultured according to the instructions on the attached product sheet.

Patient hair samples are minced and analyzed for cortisol concentrations using a Cortisol ELISA kit (CAN-C-270). Kits are handled, stored, and disposed of according to the instructions on the attached product sheets.

None of the drugs mentioned above are controlled substances.

Waste is treated as biohazard materal and decontaminated before disposal. Cells are bleached, flasks, containers and tubes are autoclaved.

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

This research is directed to understand adverse drug reactions, as well as the potiental for hair cortisol to act as a biomarker. There is a current focus on the adverse effects of ifosfamide on the kidney's of children with cancer and possible strategies for preventing this kidney toxicity. In the case of ifosmfamide, a reactive metabolite is thought to be responsible for toxicity, mainly through the mechanism of oxidative stress. Antioxidants would then serve as potential tools for attenuating this toxicity. Drug toxicity and the ability of antioxidatns to prevent this toxicity, is then assessed by in vitro experiments where cells used contain the appropritate metabolic activity for drug metabolism to the reactive metabolites. The viability of cells is determined and compared to untreated controls, giving an indication of the toxicity of the drug and the ability of antioxidants to prevent this toxicity.
There is also a focus on the use of hair coritisol as a biomarker, in particular as a marker of stress, as well as a mark of the efficacy of treatment with cortisosterioids in patients with addison's disease and cushings syndrome.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☐ NO
If YES, please give the name of the species ______
What is the origin of the microorganism(s)? ______

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

****Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: ______

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ☐ NO
(If NO, please proceed to Section 3.0)

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

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

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☒ Yes ☐ No	**HK-2**	**2**	**ATCC**
Rodent	☐ Yes ☐ No			
Non-human primate	☐ Yes ☐ No			
Other (specify)	☐ Yes ☐ No			

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

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

Additional Comments: _____

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid	**Patients treated NAC**	☐ Yes ☒ Unknown		☐1 ☒2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)	**Hair**	☐ Yes ☒ Unknown		☒1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?

Please attach a Material Safety Data Sheet or equivalent if available.
****Please attach a plasmid map.***
*****No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

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

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

Please attach a Material Safety Data Sheet or equivalent.

4.3.1 Will virus be replication defective? ☐ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☐ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☐ NO

5.0 Will genetic sequences from the following be involved?

- HIV ☒ NO ☐ YES, specify
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ☐ NO ☒ YES, specify **HPV-16 E6/E7**
- SV 40 Large T antigen ☒ NO ☐ YES
- E1A oncogene ☒ NO ☐ YES
- Known oncogenes ☒ NO ☐ YES, specify
- Other human or animal pathogen and or their toxins ☒ NO ☐ YES, specify

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

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

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

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☒ YES ☐ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used **NIH-III mice**

7.3 AUS protocol **# 2008-069 Koropatnick**

7.4 Will any of the agents listed in section 4.0 be used in live animals
☒ NO ☐ YES, specify:

7.5 Will the agent(s) be shed by the animal:
☐ YES ☒ NO, please justify:

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☐ NO

8.3 If **YES**, please specify the animal(s) used:

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

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

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☒ YES ☐ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s) **Ifosfamide**
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone **Oral LD50 Mouse = 1005 mg/kg, Oral LD50 Rat (female) = 379 mg/kg, Oral LD50 Rat (male) = 568 mg/kg, Intravenous LD50 Rat = 190 mg/kg, Intravenous LD50 Mouse = 338 mg/kg**

9.4 How much of the toxin or hormone is handled at one time*? **100mg**

9.5 How much of the toxin or hormone is stored*? **Less than 3g**

9.6 Will any biological toxins or hormones be used in live animals? ☒ YES ☐ NO
If **YES**, Please provide details: **Ifosfamide will be given to mice in doses of 60mg/kg as approved by the AUS.**

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

Additional Comments: _____

10.0 Insects

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

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

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

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

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

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

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

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

11.3 What is the origin of the plant?

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

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

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

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☒ YES, country of origin **US ATCC** ☐ NO
If **NO**, please proceed to Section 13.0

12.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☒ NO

12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? ☐ YES ☒ NO

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☒ NO

13.0 Training Requirements for Personnel Named on Form

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

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

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

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Gideon Koren ***Date:*** Sept 26, 2011

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
☒ YES, location and date of most recent biosafety inspection: **RRI 2220 and 2226**
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-RRI-003**

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO
If **YES** please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
Sqeeze the area surroudning the needlestick injury to expel blood. Wash the wound with cold running water; apply antiseptic and band aid; contact health services

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

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Gideon Koren ***Date:*** Sept 26, 2011

15.4 Additional Comments: _____

16.0 Approvals

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

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

3) Safety Officer for Institution where experiments will take place (~~if not UWO~~):
SIGNATURE: Ronald Worswick
Date: October 12, 2011

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

Special Conditions of Approval:

Designations:	HK-2
Depositors:	RA Zager
Biosafety Level:	2 [Cells Contain Papilloma viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** kidney, cortex **Tissue:** proximal tubule **Cell Type:** human papillomavirus 16 (HPV-16) transformed
Cellular Products:	alkaline phosphatase; gamma glutamyltranspeptidase; leucine aminopeptidase; acid phosphatase; cytokeratin; alpha 3, beta 1 integrin; fibronectin
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Receptors:	epidermal growth factor (EGF), expressed
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 13 D13S317: 9 D16S539: 11,12 D5S818: 12 D7S820: 10,11 THO1: 9 TPOX: 8,9 vWA: 17,18
Age:	adult
Gender:	male
Comments:	HK-2 (human kidney 2) is a proximal tubular cell (PTC) line derived from normal kidney. The cells were immortalized by transduction with human papilloma virus 16 (HPV-16) E6/E7 genes. The recombinant retrovirus vector pLXSN 16 E6/E7 containing the HPV-16 E6/E7 genes was used to transfect the ectotropic packaging cell line Psi-2. Virus produced by the Psi-2 cells was used to infect the amphotropic packaging cell line PA317 (see ATCC CRL-9078). Virus produced by the PA317 cells was used to transduce primary PTCs. Although pLXSN 16 E6/E7 also confers resistance to neomycin, selection in G418 was not used to isolate transduced clones. The cell line appears to be derived from a single cell based on Southern and FISH analysis. The E6/E7 genes are present in the HK-2 genome as determined by PCR. The cells retain a phenotype indicative of well differentiated PTCs. They are positive for alkaline phosphatase, gamma glutamyltranspeptidase, leucine aminopeptidase, acid phosphatase, cytokeratin, alpha 3,beta 1 integrin, and fibronectin. The cells are negative for factor VIII related antigen, 6.19 antigen and CALLA endopeptidase. HK-2 cells retain functional characteristics of proximal tubular epithelium such as Na+ dependent / phlorizin sensitive sugar transport and adenylate cyclase responsiveness to parathyroid, but not to antidiuretic hormone. The cells are capable of gluconeogenesis as evidenced by their ability to make and store glycogen. HK-2 cells are anchorage dependent. The cells will not grow in methylcellulose, soft agar or suspension. HK-2 cells can reproduce experimental results obtained with freshly isolated PTCs.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is provided by Invitrogen (GIBCO) as part of a kit: Keratinocyte Serum Free Medium (K-SFM), Kit Catalog Number 17005-042. This kit is supplied with each of the two additives required to grow this cell line (bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF). To make the complete growth medium, you will

Info on Cell Line(s)

Related Links

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Product Information Sheet

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

All prices are listed in U S dollars and are subject to change without notice A discount off the current list price will be applied to most cultures for nonprofit institutions in the United States. Cultures that are ordered as test tubes or flasks will carry an additional laboratory fee Fees for permits, shipping, and handling may apply

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Cell Line Designation: HK-2
ATCC® Catalog No. CRL-2190™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: kidney, cortex; proximal tubule; human papillomavirus 16 (HPV-16) transformed
Age: adult
Gender: male
Morphology: epithelial
Growth properties: adherent
DNA profile (STR analysis)

Amelogenin: X,Y
CSF1PO: 13
D13S317: 9
D16S539: 11,12
D5S818: 12
D7S820: 10,11
TH01: 9
TPOX: 8,9
vWA: 17,18

Depositors: R.A. Zager
Comments: HK-2 (human kidney 2) is a proximal tubular cell (PTC) line derived from normal kidney. The cells were immortalized by transduction with human papilloma virus 16 (HPV-16) E6/E7 genes. The recombinant retrovirus vector pLXSN 16 E6/E7 containing the HPV-16 E6/E7 genes was used to transfect the ectotropic packaging cell line Psi-2. Virus produced by the Psi-2 cells was used to infect the amphotropic packaging cell line PA317 (see ATCC CRL-9078). Virus produced by the PA317 cells was used to transduce primary PTCs. Although pLXSN 16 E6/E7 also confers resistance to neomycin, selection in G418 was not used to isolate transduced clones.
The cell line appears to be derived from a single cell based on Southern and FISH analysis.
The E6/E7 genes are present in the HK-2 genome as determined by PCR. The cells retain a phenotype indicative of well differentiated PTCs. They are positive for alkaline phosphatase, gamma glutamyltranspeptidase, leucine aminopeptidase, acid phosphatase, cytokeratin, alpha 3,beta 1 integrin, and fibronectin.
The cells are negative for factor VIII related antigen, 6.19 antigen and CALLA endopeptidase. HK-2 cells retain functional characteristics of proximal tubular epithelium such as Na+ dependent / phlorizin sensitive sugar transport and adenylate cyclase responsiveness to parathyroid, but not to antidiuretic hormone. The cells are capable of gluconeogenesis as evidenced by their ability to make and store glycogen. HK-2 cells are anchorage dependent. The cells will not grow in methylcellulose, soft agar or suspension. Cell growth is dependent on epidermal growth factor.
HK-2 cells can reproduce experimental results obtained with freshly isolated PTCs.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

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

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

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

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

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

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a **25 cm²** culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

NOTE: the cells should not be allowed to become confluent, subculture at 80% of confluence

Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.05% (w/v) Trypsin-0.53mM EDTA solution.

3. Add 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually with 5 to 15 minutes).

   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 xg for 5 to10 minutes

6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
   Subcultivation Ratio: 1:4.

7. Place culture vessels in incubators at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in ***Culture of Animal Cells, a manual of Basic Technique*** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

Two to three times weekly.

Complete Growth Medium

The base medium for this cell line is provided by Invitrogen (GIBCO) as part of a kit: Keratinocyte Serum Free Media (K-SFM), Kit Catalog Number 17005-042. This kit is supplied with each of the two additives required to grow this cell line (bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF)).

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

- 0.05 mg/ml BPE - provided with the K-SFM kit
- 5 ng/ml human r EGF - provided with the K-SFM kit.

NOTE: Do not filter complete medium.

This medium is formulated for use with a 5% CO_2 in air atmosphere.

Cryoprotectant Medium

Complete culture medium described above supplemented with 7.5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

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

References

(additional references may be available in the catalog description at www.atcc.org)

Ryan MJ et al. HK-2: **an immortalized proximal tubule epithelial cell line from normal adult human kidney**. Kidney Int. 45: 48-57, 1994 PubMed: 94172946

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 (U.S., Canada, and Puerto Rico) or 703-365-2700 (elsewhere) or by e-mail at tech@atcc.org.

Disclaimers

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

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

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

04/11

Material Safety Data Sheet

According to 9
Cortisol Saliva ELI

MSDS'

Version: *2.0*
Effective: *December 3, 2007*

1. Data on Components

Regarding components in the kit, the following products are defined as hazardous chemicals according to the Directive 67/548/EEC.
Accordingly, Material Safety Data Sheets for the following chemicals are enclosed:

2-Methyl-2H-isothiazol-3-one: At a final concentration from 0.001-0.05% is used as a preservative in the following components of the kit: *Calibrators and Control, HRP Conjugate Concentrate, Assay Buffer and Wash Buffer Concentrate.*
Sulfuric Acid: At a final concentration of 5% (v/v) is used in the *Stop Solution.*
TMB Substrate

2. General Information

The information contained in this document is believed to be correct, however, this shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship. Diagnostics Biochem Canada Inc. and ALPCO Diagnostics shall not be liable for any damages resulting from handling or from contact with the above products.

Section 1- Identification of Substance

Product Identification:

Cortisol Saliva ELISA Kit (11-CORHU-E01-SLV)
Calibrators and Control
HRP-Conjugate Concentrate
Assay Buffer
Wash Buffer Concentrate

Manufacturer:

Diagnostics Biochem Canada Inc.
1020 Hargrieve Road
London, Ontario, Canada, N6E 1P5
Tel/Fax: (519) 681-8731
E-mail: dbc@dbc-labs.com
Internet: http://www.dbc-labs.com

Distributor: 26G Keewaydin Dr.
Salem, NH 03079
Tel: (800) 592-5726

Further Information Obtainable From: Quality Management Representative (QMR).

Chemical Characterization: Preparation
Description: Mixture of substances listed below with nonhazardous additions.

Hazardous Ingredients	%	CAS Number	EC Number
2-Methyl-2H-isothiazol-3-one	0.001-0.05%	2682-20-4	220-239-6

C; R34-R43

Section 3 - Hazard Identification

Hazard Description: C Corrosive
Information concerning to particular hazards to man and environment: Irritating to eyes and skin. May cause sensitization by skin contact.
Classification System: The classification is according to the latest editions of the EU-lists, and extended by company and literature data.

Section 4 - First Aid Measures

General Information: No special measures required.
After Inhalation: Supply fresh air; consult doctor in case of complaints.
After Skin Contact: Wash off with soap and plenty of water.
After Eye Contact: Rinse opened eye for several minutes under running water.
After Ingestion: If symptoms persist consult doctor.

Section 5 - Fire Fighting Measures

Suitable Extinguishing Agents: CO_2 powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
Protective Equipment: No special measures required.

Section 6 - Accidental Release Measures

Person-Related Safety Precautions: Not required.
Measures for Environmental Protection: Dilute with plenty of water. Do not allow to enter sewers / surface or ground water.
Measures for Cleaning/Collecting: Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust).
Additional Information: No dangerous substances are released.

Section 7 - Handling and Storage

Handling:
Information for Safe Handling: Avoid contact with skin and eyes.
Information for Fire and Explosion Protection: No special measures required.

Storage:
Requirements to be met by Storerooms and Receptacles: No special requirements.
Information About Storage in One Common Storage Facility: Not required.
Further Information About Storage Conditions: None.

Storage Class:
Class According to Regulation on Flammable Liquids: Void.

Section 8 - Exposure Controls and Personal Protection Gear

Additional Information About Design of Technical Facilities: No further data; see item 7.
Ingredients with Limit Values that Require Monitoring at the Workplace: The products does not contain any relevant quantities of materials with critical values that have to be monitored at the workplace.
Additional Information: The lists valid during the making were used as the basis.

Personal Protective Equipment:
General Protective and Hygienic Measures: The usual precautionary measures are to be adhered to when handling chemicals.
Respiratory Protection: Not required.
Protection of Hands: Protective gloves.
Eye Protection: Goggles recommended during refilling.

Section 9 - Physical and Chemical Properties

Form: Liquid
Colour: Colorless
Odour: Odorless
Change in Condition
Melting Point/Melting Range: Undetermined
Boiling Point/Boiling Range: Undetermined
Flash Point: Not applicable
Self-Igniting: Product is not self-igniting.
Danger of Explosion: Product does not present an explosion hazard
Density: Undetermined
Solubility in/Miscibility With: Water: Fully miscible
pH value at 20°C: Undetermined

Section 10 – Stability and Reactivity

Thermal Decomposition: No decomposition if used according to specifications.
Dangerous Reaction: No dangerous reactions known.
Dangerous Decomposition Products: No dangerous decomposition products known.

Section 11 - Toxicological Information

Acute Toxicity
Primary Irritant Effect:
On the Skin: No irritant effect.
On the Eye: No irritant effect.
Sensitization: No sensitizing effects known.
Additional Toxicological Information: The product is not subject to classification according to the calculation method of the General EU Classification Guidelines for Preparations as issued in the latest version.

Section 12 - Ecological Information

General Notes: Generally not hazardous for water.

Section 13 - Disposal Considerations

Product:
Recommendation: Smaller quantities can be disposed of with household water.

Uncleaned Packaging:
Recommended Cleansing Agents: Water, if necessary together with cleansing agents.

Section 14 - Transport Information

Special Shipping Information: Does not constitute a hazard.

Section 15 - Regulations

Labeling According to European Labelling Guidelines: Observe the general safety regulations when handling chemicals. The product is not subject to identification regulations under EC Directives.

Section 16 - Other Information

Text of R phrases listed in section 2:
R 34 - Causes burns.
R 43 – May cause sensitization by skin contact.

This information is prepared based on our present knowledge. However, this shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship.

Prepared By: QA Department (QMR)
Contact: Mr. Ian Higgins

Section 1- Identification of Substance

Product Identification:

Cortisol Saliva ELISA Kit
Stop Solution

Manufacturer:

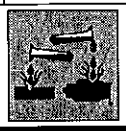
Diagnostics Biochem Canada Inc.
1020 Hargrieve Road
London, Ontario, Canada, N6E 1P5
Tel/Fax: (519) 681-8731
E-mail: dbc@dbc-labs.com
Internet: http://www.dbc-labs.com

Distributor: ALPCO Diagnostics
26G Salem, NH 03079
Tel: (800) 592-5726

Further Information Obtainable From: Quality Management Representative (QMR).

Section 2 - Data on Components

Chemical Characterization: Preparation
Description: Mixture of substances listed below with nonhazardous additions.

Hazardous Ingredients	%	CAS Number	EC Number
Sulfuric Acid	5%	7664-93-9	N/A
		C; R35	

Section 3 - Hazard Identification

Hazard Description: C Corrosive
Information concerning to particular hazards to man and environment: Causes burns.
Classification System: The classification is according to the latest editions of the EU-lists, and extended by company and literature data.

Section 4 - First Aid Measures

General Information: Immediately remove any clothing contaminated by the product.
After Inhalation: In case of unconsciousness place patient stably in side position for transportation.
After Skin Contact: Immediately wash with water and soap and rinse thoroughly.
After Eye Contact: Rinse opened eye for several minutes under running water.
After Ingestion: Do not induce vomiting. Drink plenty of water and provide fresh air. Call for a doctor immediately.

Section 5 - Fire Fighting Measures

Suitable Extinguishing Agents: CO_2, powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
Protective Equipment: No special measures required.

Section 6 - Accidental Release Measures

Person-Related Safety Precautions: Wear protective equipment. Keep unprotected persons away.
Measures for Environmental Protection: Do not allow to enter sewers / surface or ground water. Dilute with plenty of water.
Measures for Cleaning/Collecting: Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust). Use neutralizing agent. Dispose contaminated material as waste according to section 13. Ensure adequate ventilation.

Section 7 - Handling and Storage

Handling:
Information for Safe Handling: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Keep container tightly closed. Avoid contact with eyes, skin and clothing. Avoid ingestion and inhalation.
Information for Fire and Explosion Protection: No special measures required.

Storage:
Requirements to be met by Storerooms and Receptacles: No special requirements.
Information About Storage in One Common Storage Facility: Not required.
Further Information About Storage Conditions: Keep receptacle tightly sealed.

Storage Class:
Class According to Regulation on Flammable Liquids: Void.

Section 8 - Exposure Controls and Personal Protection Gear

Additional Information About Design of Technical Facilities: No further data; see item 7.
Ingredients with Limit Values that Require Monitoring at the Workplace: The products does not contain any relevant quantities of materials with critical values that have to be monitored at the workplace.
Additional Information: The lists valid during the making were used as the basis.
Personal Protective Equipment:
General Protective and Hygienic Measures: The usual precautionary measures are to be adhered to when handling chemicals. Avoid contact with eyes and skin.
Respiratory Protection: Not required.
Protection of Hands: Protective and resistant gloves.
Eye Protection: Tightly sealed goggles.

Section 9 - Physical and Chemical Properties

Form: Liquid
Colour: Colourless
Odour: Odourless
Change in Condition
Melting Point/Melting Range: Undetermined
Boiling Point/Boiling Range: Undetermined
Flash Point: Not applicable
Self-Igniting: Product is not self-igniting.
Danger of Explosion: Product does not present an explosion hazard
Density: Undetermined
Solubility in/Miscibility With: Water: Fully miscible
pH value at 20°C: <1

Section 10 – Stability and Reactivity

Thermal Decomposition/Conditions to be Avoided: No decomposition if used according to specifications.
Dangerous Reaction: No dangerous reactions known.
Incompatibilities (Concentrated Sulfuric Acid): Avoid contact with alkaline solutions, metals, metal powders, chlorates, nitrates, strong oxidizing or reducing materials, combustible organic materials and excess heat.
Dangerous Decomposition Products (Concentrated Sulfuric Acid): Toxic fumes of oxides of sulfur when heated to decomposition. Will react with water or steam to produce toxic and corrosive fumes. Reacts with carbonates to generate carbon dioxide gas, and with cyanides and sulfides to from poisonous hydrogen cyanide and hydrogen sulfide, respectively.

Section 11 - Toxicological Information

Acute Toxicity
Primary Irritant Effect:
On the Skin: Caustic effect on skin and mucous membranes.
On the Eye: Strong caustic effect.
Sensitization: No sensitizing effects known.
Additional Toxicological Information: The product shows the following dangers according to the calculation method of the General EU Classification Guidelines for Preparations as issued in the latest version: Corrosive.
Swallowing will lead to a strong caustic effect on mouth and throat and to the danger of perforation of esophagus and stomach.

Section 12 - Ecological Information

General Notes: Hazardous for water. Do not allow product to reach ground water, water course or sewage system. Must not reach sewage water or drainage ditch undiluted or unneutralized. Danger to drinking water even if small quantities leak into the ground.

Section 13 - Disposal Considerations

Product:
Recommendation: Smaller quantities can be disposed of with household water.

Uncleaned Packaging:
Recommended Cleansing Agents: Water, if necessary together with cleansing agents.

Section 14 - Transport Information

Special Shipping Information: Does not constitute a hazard.

Section 15 - Regulations

Labelling According to European Labelling Guidelines: Observe the general safety regulations when handling chemicals. The product has been classified and marked according to EC Directives.
Code Letter and Hazard Designation of Product: C Corrosive
Risk Phrases:
R 34 – Causes burns.
Safety Phrases:
S 26 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 36/37/39 – Wear suitable protective clothing, gloves and eye/face protection.
S 45 – In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S 60 – This material and its container must be disposed of as hazardous waste.

Section 16 - Other Information

This information is prepared based on our present knowledge. However, this shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship.

Prepared By: QA Department (QMR)
Contact: Mr. Ian Higgins

Section 1- Identification of Substance

Product Identification:

Cortisol Saliva ELISA Kit
TMB Substrate

Manufacturer:

Diagnostics Biochem Canada Inc.
1020 Hargrieve Road
London, Ontario, Canada, N6E 1P5
Tel/Fax: (519) 681-8731
E-mail: dbc@dbc-labs.com
Internet: http://www.dbc-labs.com

Distributor:

ALPCO Diagnostics
26G Keewaydin Dr.
Salem, NH 03079
Tel: (800) 592-5726

Further Information Obtainable From: Quality Management Representative (QMR).

Section 2 - Data on Components

Chemical Characterization: Preparation
Description: Mixture of substances listed below with nonhazardous additions.
Hazardous Ingredients: Void.

Section 3 - Hazard Identification

Hazard Description: N/A
Information concerning to particular hazards to man and environment: N/A
Classification System: The classification is according to the latest editions of the EU-lists, and extended by company and literature data.

Section 4 - First Aid Measures

General Information: Immediately remove any clothing contaminated by the product.
After Inhalation: Remove the exposed person to fresh air and support breathing, if necessary. Obtain medical attention.
After Skin Contact: Remove contaminated clothing and wash effected skin with soap and water. Seek medical attention if irritation is noted.
After Eye Contact: Immediately flush eyes thoroughly with fresh water. Remove contact lenses, if any, and seek medical attention.
After Ingestion: Wash out mouth immediately provided that person is conscious. Obtain medical attention.

Section 5 - Fire Fighting Measures

Suitable Extinguishing Agents: CO_2 powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
Protective Equipment: Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section 6 - Accidental Release Measures

Person-Related Safety Precautions: Wear suitable protective equipment to prevent inhalation and skin or eye contact.
Measures for Environmental Protection: No special measures required.
Measures for Cleaning/Collecting: Clean up small spills with a sponge or mop and water. Larger volumes can be absorbed with sand or vermiculite.
Additional Information: N/A

Section 7 - Handling and Storage

Handling:
Information for Safe Handling: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Keep container tightly closed. Avoid contact with eyes, skin and clothing. Avoid ingestion and inhalation.
Information for Fire and Explosion Protection: No special measures required.

Storage:
Requirements to be met by Storerooms and Receptacles: Store refrigerated (~4-8˚C) in a dry, well-ventilated area away from incompatible substances (see section 10). Protect from exposure to sunlight.
Information About Storage in One Common Storage Facility: Not required.
Further Information About Storage Conditions: None.

Storage Class:
Class According to Regulation on Flammable Liquids: Void.

Section 8 - Exposure Controls and Personal Protection Gear

Additional Information About Design of Technical Facilities: No further data; see item 7.
Ingredients with Limit Values that Require Monitoring at the Workplace: The products does not contain any relevant quantities of materials with critical values that have to be monitored at the workplace.
Additional Information: The lists valid during the making were used as the basis.

Personal Protective Equipment:
General Protective and Hygienic Measures: The usual precautionary measures are to be adhered to when handling chemicals. Avoid contact with eyes and skin.
Respiratory Protection: Not required.
Protection of Hands: Protective gloves.
Eye Protection: Goggles recommended during refilling.

Section 9 - Physical and Chemical Properties

Form: Liquid
Colour: Colourless
Odour: Odourless
Change in Condition
Melting Point/Melting Range: N/A
Boiling Point/Boiling Range: 81˚C
Flash Point: 187˚F (Closed cup)
Self-Igniting: Product is not self-igniting.
Danger of Explosion: Product does not present an explosion hazard
Density: Undetermined
Solubility in/Miscibility With: Water: Fully miscible
pH value at 20℃: Undetermined

Section 10 – Stability and Reactivity

Thermal Decomposition: During heating, carbon monoxide and nitrogen oxides may be produced.
Incompatibilities: Avoid contact with strong oxidizing agents. Protect from exposure to sunlight.
Dangerous Decomposition Products: Toxic fumes of oxides of carbon monoxide and nitrogen oxide.

Section 11 - Toxicological Information

Acute Toxicity
Primary Irritant Effect:
On the Skin: Irritant to mucous membranes.
On the Eye: Irritant.
Sensitization: No sensitizing effects known.
Additional Toxicological Information: The product is not subject to classification according to the calculation method of the General EU Classification Guidelines for Preparations as issued in the latest version.

Section 12 - Ecological Information

General Notes: Slightly hazardous for water. Do not allow product to reach ground water, water course or sewage system. Must not reach sewage water or drainage ditch undiluted.

Section 13 - Disposal Considerations

Product:
Recommendation: Smaller quantities can be disposed of with household water.

Uncleaned Packaging:
Recommended Cleansing Agents: Water, if necessary together with cleansing agents.

Section 14 - Transport Information

Special Shipping Information: Does not constitute a hazard.

Section 15 - Regulations

Labelling According to European Labelling Guidelines: Observe the general safety regulations when handling chemicals. The product is not subject to identification regulations under EC Directives.
Safety Phrases:
S 60 – This material and its container must be disposed of as hazardous waste.

Section 16 - Other Information

This information is prepared based on our present knowledge. However, this shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship.

Prepared By: QA Department (QMR)
Contact: Mr. Ian Higgins

Cortisol (Saliva) ELISA

For the quantitative determination of cortisol in human saliva

Please read carefully due to Critical Changes, e.g., additional control

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	11-CORHU-E01-SLV
Size:	96 wells
Version:	4.0 10/26/2010 - ALPCO February 14, 2011

ALPCO Diagnostics

26G Keewaydin Drive • Salem, NH 03079
Phone: (800) 592-5726 • Fax: (603) 898-6854
www.alpco.com • Email: web@alpco.com

INTENDED USE

For the quantitative determination of Cortisol by enzyme immunoassay in human saliva.
For *Research Use Only. Not For Use In Diagnostic Procedures.*

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and samples) and an enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stop solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed is inversely proportional to the concentration of cortisol in the sample. A set of standards is used to plot a standard curve from which the amount of cortisol in samples and controls can be directly read.

INTRODUCTION

Cortisol is the most abundant circulating steroid and the major glucocorticoid secreted by the adrenal cortex. Cortisol is physiologically effective in blood pressure maintenance and anti-inflammatory activity. It is also involved in calcium absorption, gluconeogenesis as well as the secretion of gastric acid and pepsin. It is increased under stress situation, physical exercise and external administration of ACTH. Measurement of cortisol levels in general can be used as an indicator of adrenal function and the differential diagnosis of Addison's and Cushing's diseases as well as adrenal hyperplasia and carcinoma.
Most circulating cortisol is bound to cortisol binding globulin or transcortin and albumin. The free cortisol, which is considered the active part of blood, is about 1-2%. In the absence of appreciable amounts of the cortisol binding proteins in saliva, salivary cortisol is considered to be free and shows a diurnal rhythm with the highest levels in the morning and the lowest levels at night.

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.

11. When dispensing the substrate and stop solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of cortisol in human saliva. The kit is not calibrated for the determination of cortisol in serum, plasma or other specimens of human or animal origin.
2. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
3. Only calibrator A may be used to dilute any high saliva samples. The use of any other reagent may lead to false results.
4. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in subjects regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a subject's background including the frequency of exposure to animals/products if false results are suspected.

SAFETY CAUTIONS AND WARNINGS
POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 1 ml of saliva is required per duplicate determination. Collect 4-5 ml of saliva into a clean glass tube (Salivette by Sarstedt may be used) without force or inducement and before eating, drinking or brushing the teeth. Simply rinse the mouth with water before collection. Do not use blood-contaminated specimens. Store samples at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

Specimen tubes are to be placed into a freezer and allowed to freeze. When ready to use, the specimens are to be thawed and centrifuged. The supernatants are to be collected and poured into freshly labeled tubes.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100, 150 and 300 µl
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Bench top centrifuge
6. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).

REAGENTS PROVIDED

1. Rabbit Anti-Cortisol Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.
Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

2. Cortisol-Horseradish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.
Contents: Cortisol-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 300 µl/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:51 in assay buffer before use (eg. 40 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µl of HRP in 12 ml of assay buffer. Discard any that is left over.

3. Cortisol Saliva Calibrators - Ready To Use.
Contents: Six vials containing cortisol in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of cortisol.
*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 ng/ml	2.0 ml
Calibrator B	1 ng/ml	0.6 ml
Calibrator C	3 ng/ml	0.6 ml
Calibrator D	10 ng/ml	0.6 ml
Calibrator E	30 ng/ml	0.6 ml
Calibrator F	100 ng/ml	0.6 ml

Storage: Refrigerate at 2-8°C
Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Controls - Ready To Use.
Contents: Two vials containing cortisol in a protein-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of cortisol. Refer to vial labels for the acceptable range.
Volume: 0.6 ml/vial
Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate - Requires Preparation.
Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

6. Assay Buffer - Ready To Use.
Contents: One vial containing a protein-based buffer with a non-mercury preservative.
Volume: 15 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

7. TMB Substrate - Ready To Use.
Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume: 16 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

8. Stop Solution - Ready To Use.
Contents: One vial containing 1M sulfuric acid.
Volume: 6 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

ASSAY PROCEDURE
Specimen Pretreatment:
Freezing and Centrifugation.

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the cortisol-HRP conjugate and wash buffer.

2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.

3. Pipette 50 µl of each calibrator, control and specimen sample into correspondingly labeled wells in duplicate.

4. Pipette 100 µl of the conjugate working solution into each well (The use of a multichannel pipette is recommended).

5. Incubate on a plate shaker (approximately 200 rpm) for 45 minutes at room temperature.

6. Wash the wells 3 times with 300 µl of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).

7. Pipette 150 µl of TMB substrate into each well at timed intervals.

8. Incubate on a plate shaker for 15-20 minutes at room temperature (or until calibrator A attains dark blue color for desired OD).

9. Pipette 50 µl of stop solution into each well at the same timed intervals as in step 7.

10. Read the plate on a microwell plate reader at 450nm within 20 minutes after addition of the stop solution.

*If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of the samples or controls.

CALCULATIONS

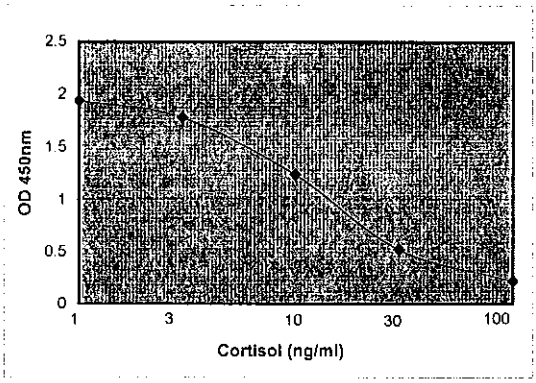
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 100 ng/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Calibrator	OD 1	OD 2	Mean OD	Value (ng/ml)
A	2.241	2.133	2.187	0
B	1.965	1.914	1.940	1
C	1.757	1.799	1.778	3
D	1.221	1.254	1.238	10
E	0.540	0.502	0.521	30
F	0.222	0.216	0.219	100
Unknown	0.287	0.283	0.285	63

TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the Cortisol (Saliva) ELISA kit is **1.0 ng/ml.**

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Cortisol (Saliva) ELISA kit with cortisol cross-reacting at 100%.

Steroid	%Cross Reactivity
Cortisol	100
Prednisolone	13.6
Corticosterone	7.6
Deoxycorticosterone	7.2
Progesterone	7.2
Cortisone	6.2
Deoxycortisol	5.6
Pednisone	5.6
Dexamethasone	1.6

No cross-reaction was detected with DHEAS and Tetrahydrocortisone.
Please note that there is an observed cross-reactivity of 13.6% with prednisolone. Since prednisone is converted to prednisolone in vivo, caution must be exercised when assaying the cortisol levels of individuals undergoing either therapy.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV%
1	6.6	0.68	10.3
2	24.8	1.98	8.0
3	52.4	3.40	6.5

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV%
1	6.3	0.63	9.8
2	23.7	2.06	8.7
3	51.8	3.37	6.5

RECOVERY

Spiked samples were prepared by adding defined amounts of cortisol to three patient saliva samples (1:1). The results (in ng/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	6.28	-	-
+ 1.0	4.14	3.64	113.7
+ 10	9.05	8.14	111.2
+ 100	61.85	53.14	116.4
2 Unspiked	8.03	-	-
+ 3.0	6.05	5.52	109.6
+ 30	20.64	19.02	108.5
+ 100	52.20	54.02	96.6
3 Unspiked	6.98	-	-
+ 3.0	5.38	4.99	107.8
+ 10	8.76	8.49	103.2
+ 30	19.00	18.49	102.8

LINEARITY

Three patient saliva samples were diluted with calibrator A. The results (in ng/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	18.18	-	-
1:2	10.32	9.09	113.5
1:4	5.09	4.55	112.1
1:8	2.20	2.27	96.7
2	49.89	-	-
1:2	28.03	24.95	112.3
1:4	13.29	12.47	106.6
1:8	7.97	7.24	110.1
3	68.53	-	-
1:2	34.27	31.49	91.9
1:4	17.13	13.81	80.6
1:8	8.57	7.48	87.3

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Random male and female samples were taken in the early morning and had an absolute range of:

5 - 21.6 ng/ml

REFERENCES

1. Brock P.,et al., Clinical Chemistry 24/9:1595, 1978.
2. Morris R., Annals of Clinical Biochemistry 15:178, 1978.
3. Silver A.C., et al., Clinical Chemistry 29:1869, 1983.
4. Vecsei P., et al., Experimentia 28:1104, 1972.
5. Abraham G.E. et al., Anal Lett. 5:757, 1972.
6. Gomez-Sanchez C., etal., J. Lab. Clin.Med 89:902, 1977.
7. Demeris L.M., et al., Clin. Biochem. 10:104, 1977.
8. Poland R.E., et al., Life Sci. 30:177, 1982.
9. Peters J.R., et al., Clin Edocrinol. 17:583, 1982.
10.Papanicolaou,D.A. et al J. Cli Endocrinol Metab 87(10) 4515-4521.
11.Check, J.H., et al, Falsely elevated steroidal assay levels related to heterophile antibodies against various animal species. Gynecol Obstet Invest 40:139-140, 1995.

Baxter

Material Safety Data Sheet

Info on Toxin(s)

MSDS Number: 1118563
Revision date: 02/10/2009
Print date: 02/10/2009

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

MSDS Number: 1118563
Product name: Ifosfamide for Injection
Product Codes: NDC 10019-925-01, NDC 10019-926-02, NDC 0338-3991-01, NDC 0338-3993-01
Synonyms: IFEX (Ifosfamide for Injection, USP); Holoxan; Tronoxal
Chemical Family: Antineoplastic Agent
Product Type: Regulated Prescription Drug
Container Information: 1 and 3 gram vials
Product Use: Pharmaceutical.

Supplier:
BAXTER HEALTHCARE CORPORATION
DEERFIELD, ILLINOIS 60015
(800) 422-9837 or (847) 948-4770

Emergency telephone number: PROSAR: USA (888) 990-0996 OUTSIDE USA (615)917-6114
CHEMTREC: USA (800) 424-9300 OUTSIDE USA (743)741-6089

2. COMPOSITION/INFORMATION ON INGREDIENTS

Component	Weight %	UN Number:	Classification:
Ifosfamide 3778-73-2	100	None	None

3. HAZARDS IDENTIFICATION

Emergency overview: WARNING! HARMFUL IF SWALLOWED. MAY CAUSE CANCER. POSSIBLE REPRODUCTIVE HAZARD THAT MAY CAUSE ADVERSE REPRODUCTIVE EFFECTS. DEVELOPMENTAL HAZARD THAT MAY ADVERSLEY EFFECT THE DEVELOPING FETUS. Irritating to eyes. Can be absorbed through the skin. Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin, and if swallowed.

Principle routes of exposure: Absorbed through skin. Eye contact. Ingestion. Inhalation.

Inhalation: Toxic: danger of serious damage to health by prolonged exposure through inhalation. Irritating to respiratory system.

Ingestion: Harmful if swallowed. Toxic: danger of serious damage to health by prolonged exposure if swallowed. Gastrointestinal effects (may include nausea/vomiting, abdominal pain, diarrhea, dry mouth, colic). Nervous System effects (may include ataxia, tremor, disturbance of speech, lethargy, headache, dizziness). Renal function effects (may include increased levels of creatinine, BUN, renal failure). Hemorrhagic cystitis. Hair loss. May cause allergic reaction.

3. HAZARDS IDENTIFICATION

Skin contact: Toxic: danger of serious damage to health by prolonged exposure with skin. Can cause skin irritation.

Eye contact: Irritating to eyes.

4. FIRST AID MEASURES

Inhalation: If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical attention.

Skin contact: In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Get medical attention

Ingestion: Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Seek medical attention.

Eye contact: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention

Notes to physician: See patient package insert in shipping carton for complete information.

5. FIRE FIGHTING MEASURES

Suitable extinguishing media: Use foam or all purpose dry chemicals to extinguish.
Special protective equipment for firefighters: Fire fighters should wear positive pressure self-contained breathing apparatus (SCBA) and full turnout gear.
Specific methods: No information available
Flash point: Not determined
Autoignition temperature: Not available
Flammable limits in air-lower (%): Not available
Flammable limits in air-upper (%): Not available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions: Keep unnecessary personnel away. Use suitable protective equipment (Section 8). Follow all fire fighting procedures (Section 5).

Environmental precautions: Do not discharge into soil or water.

Methods for cleaning up: If emergency personnel are unavailable, contain spilled material. Absorb with vacuum cleaner approved for carcinogenic substances. Avoid production of dust. Place spilled material in an appropriate container for disposal. Clean contaminated surface thoroughly.

7. HANDLING AND STORAGE

Handling:
Technical measures/precautions: Do not ingest. Avoid skin contact. Avoid eye contact. Wash thoroughly after handling.

Storage:

7. HANDLING AND STORAGE

Technical measures/Storage conditions: Keep containers tightly closed in a cool, well-ventilated place. Store at room temperature between 20°C and 25°C (68°F and 77°F). Protect from temperatures above 30°C (86°F).

Incompatible products: No special restrictions on storage with other products.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Occupational Exposure Limits

Component	OSHA- Time Weighted Average:	OSHA- Short Term Exposure Limit:	OSHA- Ceiling Limits	ACGIH- Time Weighted Average:	ACGIH- Short Term Exposure Limit:	ACGIH- Ceiling Limit Value:
Ifosfamide 3778-73-2	None	None	None	None	None	None

Engineering measures: Provide general or local exhaust ventilation. Open handling must be limited.

Personal protective equipment:

Respiratory protection: No personal respiratory protective equipment normally required.

Hand protection: Use chemical resistant, impervious gloves.

Skin and body protection: Disposable close-front gown or coveralls.

Eye protection: Goggles, face shield, or other full-face protection if potential exists for direct exposure to dust.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical state: Solid.
Appearance: Crystalline
Color: White
Odor: Odorless
pH: 4.0 - 7.0
Molecular weight: 261.1
Boiling point/range: Not available.
Melting point/range: 48-51 °C
Density: Not available.
Vapor pressure: Not available.
Evaporation rate: Not available.
Solubility: Soluble in water.
Partition coefficient (n-octanol/water): Not available.
Viscosity: Not available.
% Volatile by Volume: Not available.

10. STABILITY AND REACTIVITY

Stability: Stable under recommended storage conditions.

10. STABILITY AND REACTIVITY

Polymerization: Not applicable

Hazardous decomposition products: Halogenated hydrocarbons. Organic nitrogenous products of decomposition. Organic compounds of phosphoric compounds.

Materials to avoid: Acids. Alkali metals. Oxidizing agents.

11. TOXICOLOGICAL INFORMATION

Component	LD50s and LC50s
Ifosfamide 3778-73-2	Oral LD50 Mouse = 1005 mg/kg Oral LD50 Rat (female) = 379 mg/kg Oral LD50 Rat (male) = 568 mg/kg Intravenous LD50 Rat = 190 mg/kg Intravenous LD50 Mouse = 338 mg/kg

Acute toxicity: Gastrointestinal effects (may include nausea/vomiting, abdominal pain, diarrhea, dry mouth, colic). Nervous System effects (may include ataxia, tremor, disturbance of speech, lethargy, headache, dizziness, blurred vision). Renal function effects (may include increased levels of creatinine, BUN, renal failure). Hemorrhagic cystitis. Hair loss. May cause allergic reactions. Irritating to skin, eyes, and respiratory system.

Chronic toxicity: No data is available on the product itself.

Carcinogenic effects: May be carcinogenic. Carcinogenic category 2. See patient package insert for additional information.

Mutagenic effects: May be mutagenic. Mutagenic category 2. See patient package insert for additional information.

Reproductive toxicity: May impair fertility. May be fetotoxic. Reproductive category 2. See patient package insert for additional information.

FDA Pregnancy Catagory D

12. ECOLOGICAL INFORMATION

Environmental properties:

Component	Ecotoxicity - Water Flea Data	Fish Species Ecotoxicity	Ecotoxicity - Freshwater Algae Data	Ecotoxicity - Microtox Data
Ifosfamide 3778-73-2	None.	None.	None.	None.

Ecotoxicity effects: LC50 (96h): >1000 mg/l; NOEC (96 h): > 555 mg/l Salmo gairdneri; EC50 (48 h): 162 mg/l Daphnia; NOEC (48 h): 100 mg/l Daphnia magna.

Bioaccumulation: Not easily biodegradable.

13. DISPOSAL CONSIDERATIONS

Waste Classification: Hazardous waste.

Waste from residues / unused products: Waste disposal must be in accordance with appropriate Federal, State, and local regulations.

14. TRANSPORT INFORMATION

DOT:

DOT shipping name:	None
UN Number:	None
DOT Packing Group:	None
DOT Hazard class:	None

TDG (Canada):

TDG Proper shipping name:	None
TDG UN/NA Number:	None
TDG Packing Group:	None
TDG Hazard class:	None

ADR/RID:

ADR Official Transport Name:	Not Available.
ADR Proper shipping name:	Not Available.
ADR UN/NA number:	Not Available.
ADR Hazard Class:	Not Available.

ICAO / IATA:

IATA Proper shipping name:	None
IATA UN NUMBER	None
IATA Primary Hazard	None
IATA Packing group:	None
ICAO ERG Code:	None

IMO / IMDG:

IMDG Proper Shipping Name:	Not Available.
IMDG Hazard Class and Division:	Not Available.
IMDG Packing Group:	Not Available.
IMDG Subsidiary Risks:	Not Available.

15. REGULATORY INFORMATION

U.S. Regulations:

TSCA Inventory List - The product is exempt from TSCA, it is FDA Regulated

OTHER REGULATIONS:

Japanese Inventory (ENCS) This product does not comply with JPENCS

Component	Weight %	RCRA Status:	CERCLA Reportable Quantity:	CERCLA/SARA - 302 Ext. haz. substances:	Listed as Sara 313 title III:
Ifosfamide	100	Not Listed	Not Listed	Not Listed	Not Listed

STATE REGULATIONS:

Component	California Prop 65:	Minnesota Right-To - Know:	Florida Right-to-Know Reporting List:	Rhode Island Right-to-Know List:	Massachusetts Right-to-Know List:	Pennsylvania Right-to-Know:	New Jersey Right-to-Know:
Ifosfamide	developmental toxicity, initial date 7/1/90	Not Listed	Not Listed	Not Listed	Carcinogen; Extraordinarily hazardous	Not Listed	Not Listed

This product contains component(s) known to the State of California to cause cancer or reproductive/developmental harm.

CANADIAN REGULATIONS:

Canada DSL Inventory List - This product does not comply with DSL

EU EINECS List - This product complies with EINECS

Risk Phrases:
R22 - Harmful if swallowed.
R36 - Irritating to eyes.
R45 - May cause cancer.
R46 - May cause heritable genetic damage.
R48/23/24/25 - Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed.
R61 - May cause harm to the unborn child.

Safety Phrases:
S53 - Avoid exposure - obtain special instructions before use.
S15 - Keep away from heat.
S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S45 - In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

16. OTHER INFORMATION

This data sheet contains changes from the previous version in section(s)?:
Additional product codes. Changes to Section 1. Changes to Section 8.

Additional information:
NOEC = No observable effect concentration

Prepared by: Technology Resources, Baxter Healthcare Corporation

To the best of our knowledge, the information contained herein is accurate. However, neither the above named supplier nor any of its subsidiaries assumes any liability whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

End of safety data sheet:

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Ifosfamide
Proposed Use Dose:	100000 µg
Proposed Storage Dose:	3000000 µg
LD $_{50}$ (species):	190000 µg

Calculation:

190000 µg/kg x 50 kg/person

Dose per person based on LD $_{50}$ in µg = 9500000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = ***950000***

Comments/Recommendations: