EDVOTEK®

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EDVO-Kit

1001

Eukaryotic Cell Biology Using Insect Cell Culture

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to introduce students to the simple and inexpensive insect cell culture system. Basics of cell culture will be introduced as a platform for studies on viability and cell growth.

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Funded in part by NIH/ R43 MD005202 from the National Institute on Minority Health and Health Disparities

This experiment is designed for 6 student groups.

Please conduct the experiment within one week of receipt of kit. If not, place the OptiCell chamber containing the insect cells at room temperature in a draft-free environment (shoe box) to avoid any temperature fluctuations. In addition, store the insect cell growth media in the refrigerator (4° C).

Experiment Components

		Storage
A	Insect Cells (Sf9)	Room Temperature
B	Insect Cell Media	4° C Refrigerator
C	Trypan Blue Dye	Room Temperature
D	Giemsa stain	Room Temperature
E	Phosphate Buffered Saline (PBS)	Room Temperature

- Cell culture flasks (T-25) 25 cm^2 (Sterile)
- Cell culture dishes (60 mm) (Sterile)
- Cell Counting chambers
- Large transfer pipets (Sterile)
- Small transfer pipets
- 10 ml and 25 ml pipets
- 15 ml Conical Bottom Tubes
- 50 ml Conical Bottom Tubes (Sterile)
- 1.5 ml microcentrifuge tube

Requirements

- Covered large plastic container for use as incubation chamber or cardboard box with cover (the EDVOTEK box will work to grow cultures)
- 70% Ethanol in spray bottles
- Methanol
- Pipette pump or bulb
- Inverted phase contrast/ bright field microscope (Cells can be viewed with an upright student microscope, see additional notes in the Instructor's Guide)
- 10 ml Syringe and needle or 1000 µl micropipet and tips
- Marking pens
- Safety goggles and disposable laboratory gloves
- Face mask

Background Information

Animal cell culture is the process by which hundreds of eukaryotic cells, from dozens of species, have been stabilized and grown in vitro (Landecker, 2007). Cell culture continues to play a critical role in biotechnology, pharmaceutical, and basic life science research. In science education, cell culture provides a platform for teaching essential cell biology concepts, such as cellular architecture, cellular behavior, and alterations that occur in disease states. In pharmaceutical research, cell culture continues to become an even more critical tool, replacing prokaryotic systems with fully automated, high-throughput, drug-screening systems. Understanding biological processes at the cellular level provides the opportunity to apply various aspects of biotechnology in animals and eventually, in humans. Cell culture studies minimize the use of vertebrate animals (thus reducing costs and completely avoiding any animal suffering), and can provide biologically meaningful answers in reasonable time frames, including efficacy studies of novel drugs, allowing the rapid identification of those that show promise, greatly facilitating development of the next generation of diagnostics and therapeutics.

To date cell culture has not been widely available for laboratory teaching activities in high schools. Although there are some published classroom cell culture experiments, there are still very few affordable educational laboratory supplies to serve the needs for teaching biology, cellular physiology, or multi-disciplinary science courses. In classroom settings, cell culture can also be used to demonstrate the biological effects of environmental agents on overall cell health and various cellular processes, including apoptosis, mitosis, and differentiation. Other biotechnology applications of cell culture for classroom experiments include models for cancer and other diseases, the effect of drugs on cell biology, and the production of high value gene products.

Insect cell culture was originally spawned by interest in developing counter measures for agricultural pests, and ovarian cells from the caterpillar-related armyworm *Spodoptera frugiperda* (Sf9), have emerged as an excellent model system for examining cellular processes that occur in higher eukaryotes (Rhee et al., 2002; Aparna et al., 2003; Mohan et al., 2003) and is now widely used to express recombinant proteins at high levels (Kulakosky et al., 2003; Wu et al., 2004; Pijlman et al., 2006; Gatehouse et al., 2008).

The advantage of insect cell culture for use in education is that cells can be grown without the use of expensive and difficult-to-maintain incubators that strictly regulate temperature, humidity, and CO_2 as required for culturing of mammalian cells. Insect cell cultures can be grown in culture dishes at room temperature, thus making this ideal for high school classroom activities.

In the past five years, the biotechnology industry has experienced a shortage of qualified entry-level and mid-level scientists. Meeting this demand requires a new generation of technicians and scientists possessing a diverse set of life science skills (Timerman, 2007). In the context of commercial applications, cell culture provides a large-scale ability to produce important products such as monoclonal antibodies and recombinant proteins that can be used in medicine that can be rapidly purified for various biomedical uses. This industry is expected to continue to be a growth industry and an engine of the U.S. economy. A large number of high-paying opportunities will be available in this industry for today's students who will be educated in these disciplines (Timerman, 2007).

In this experiment, students will acquire some basic practical skills for manipulation and growth of insect cell culture, routine maintenance and examination of cells, as well as cell counting and cell viability determinations.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to introduce students to the simple and inexpensive insect cell culture system. Basics of cell culture will be introduced as a platform for studies on viability and cell growth.

LABORATORY NOTEBOOK RECORDING

Address and record the following in your laboratory notebook or on a separate worksheet.

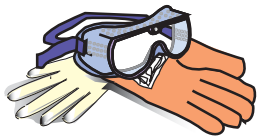
Before starting the experiment:
- Write the objectives of the laboratory experiment.
- Write a hypothesis where you predict experimental outcomes.
- Record the detailed procedures performed in the experiment.

During the experiment:
- Record (draw) your observations and photograph the results as needed.
- Prepare tables or figures showing your results.

Following the experiment:
- Formulate an explanation for the results.
- List possible sources of error if any.
- Determine what could be changed in the experiment if the experiment were to be repeated.
- Write your conclusions based on the results.

Student Experimental Procedures

LABORATORY SAFETY



1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Wearing a laboratory coat is advised as the kit uses stains that can damage clothing and stain skin.
3. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
4. DO NOT MOUTH PIPET REAGENTS - USE PIPETTORS.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.
6. Properly dispose materials after completing the experiment:

    A. Wipe down the lab bench with a 10% bleach solution, 70% Ethanol or a laboratory disinfectant.

    B. All materials, including culture dishes, pipets, transfer pipets, and tubes, that come in contact with cells should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:

    - Autoclave at 121° C for 20 minutes.
      Close flask caps and remove media from dishes before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium from spilling into the sterilizer chamber.

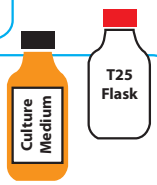
    - Soak in 10% bleach solution.
      Immerse dishes, open tubes and other contaminated materials Into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach. At the end of each day remove and discard gloves and wash hands thoroughly.

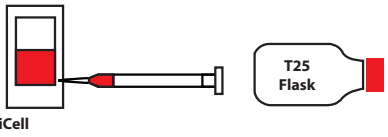
Student Experimental Procedures

EXPERIMENT FLOW CHART

1. Prepare Cell Culture Area:
   - Spray surface with 70% ethanol.

2. Prepare the Insect Cell Medium:
   - Add Insect Cell medium to the T25 flask.

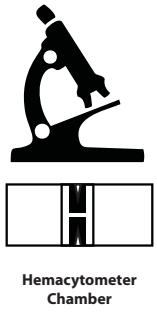




3. Initiate Insect Cell Culture (INSTRUCTOR):
   - Detach cells from cell chamber by gently shaking.
   - Using a syringe with the needle, transfer the cells from chamber to the T25 flask.
   - Place and incubate the T25 flask containing the Insect Cells inside a plastic container with lid at room temperature.

4. Basic Techniques before starting Cell Culture of Insect Cells (STUDENTS):
   - Basic aseptic technique: Learn how to work in a sterile environment
   - Prepare an incubator for the cells: Use a light-tight plastic container (box) to grow the cells.

5. Experiments performed using Insect Cells (STUDENTS):
   - Cell Examination: Observe health, morphology and confluency of Insect Cells under phase contrast microscope. Analyze the growth phase. Draw or take pictures of cells. Record results in Data Subculture Record.
   - Cell Maintenance: Feed or subculture the cells depending on their confluence. Record results in Data Subculture Record.
   - Counting Assay: Using the hemacytometer, count the cells under the microscope and prepare a growth curve. Record results in Data Subculture Record.
   - Viability Assay: Add Trypan blue to the cells and count the live and dead cells using the hemocytometer. Record results in Data Subculture Record.
   - Staining Assay: Stain the cells with Giemsa and observe under the microscope to observe finer details (e.g. nucleous and cytoplasm).



Hemacytometer Chamber

Student Experimental Procedures

Experiment Procedure

I. BASIC ASEPTIC TECHNIQUE

Successful cell culture depends heavily on keeping the cells free from contamination by microorganisms such as bacteria, fungi, and viruses. All materials that come into contact with the cell culture must be sterile and manipulations must not allow any direct link between the cell culture and its non-sterile surroundings.

Prepare a designated clean bench/cell culture area. Start with a completely clear surface. Follow the procedures to maintain aseptic conditions in all the pre-lab and lab experiments.

Materials: Spray bottle with 70% Ethanol, large plastic container or cardboard box, aluminum foil.

NOTE: Don't forget the Basic Aseptic Technique!

A. Learning Basic Aseptic Techniques

Swabbing

1. Spray and swab down bench surface with 70% Ethanol.
2. Bring components of cell culture media from the refrigerator and freezer, swab bottles or tubes with 70% Ethanol. Bring only those items you require for a particular procedure to the cell culture area. Place those that you will need first closest to you.
3. Place pipettes at the rear or side of the work surface in an accessible position. Collect the T-25 cell culture flasks that you will need.
4. Arrange your work area (a) to have easy access to all of it without having to reach over one item to get at another and (b) to leave a wide, clear space in the center of the bench. If you have too many things close to you, you will inevitably brush the tip of a sterile pipette against a non-sterile surface.
5. Mop up any spillage immediately and swab with 70% Ethanol to minimize contamination spreading to your cell culture.
6. On completion of a specific procedure, remove stock solutions from work surface, keeping only the bottles that you will require for the next.

Personal Hygiene

7. Lab gowns and face masks are strongly recommended. Tie back long hair. Talking should be kept to a minimum.
8. Disposable gloves should be worn and sprayed with 70% Ethanol as needed.

Student Experimental Procedures

Pipeting

9. Use disposable sterile plastic pipettes (10 ml and 25 ml) together with portable pipet aids. Make sure that the pipet aid fits comfortably in your hand and is easy to operate with one hand. (Transfer pipets are provided for use in all steps of the experiments.)

10. Work within your range of vision. Insert a pipette in the pipet aid with the tip of the pipette pointing away from you. Ensure that it is in your line of sight continuously and not hidden by your arm. Make sure the pipet is tilted away from you, or to the side, so your hand is never over an open bottle or flask.

Handling Bottles and Flasks

11. Bottles should not be vertical when open, but should be kept at an angle as shallow as possible without risking spillage. Do not leave reagent/media bottles open and do not work immediately above an open bottle or flask.

12. Culture flasks should be laid down horizontally when open and held at an angle during manipulations.

Pouring

13. Do not pour from one sterile container into another unless the bottle you are pouring from will be used once only and will deliver all its contents (premeasured) in one transfer. Pouring causes the formation of a bridge of liquid between the inside and outside of the bottle, which could result in a contaminated bottle.

At the End of the Experiment

14 Remove all used and unused pipettes, flasks, etc. from the work area when and swab down the work surface with 70% Ethanol at the end of the experiment.

15. Return all media and stock solutions in the cold room or refrigerator.

Student Experimental Procedures

B. Preparing Sterile Incubation Chambers

Incubators are widely used in microbiology and cell biology to culture bacteria and eukaryotic cells. The incubators are employed mainly to maintain control of temperature, humidity, and other conditions such as carbon dioxide and oxygen content of the atmosphere inside. The advantage of working with Insect Cells is that they can be grown at room temperature and do not require a complicated growth environment. The culture flasks can be incubated in a plastic light box at room temperature.

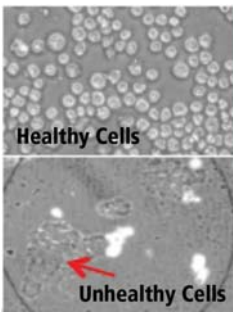
Select an appropriate sized plastic container or cardboard box with lid.

1. Cover the container with aluminum foil to avoid the light (Insect Cells do not grow under direct light).

2. Swab the inside of the container with 70% Ethanol. Allow to dry before placing plates for incubation.

3. After placing the plates/flasks inside the container, find a draft-free area in the lab that will hold a temperature between 20-25° C. (One ideal place is a cupboard or desk drawer.)

Student Experimental Procedures



II. EXAMINATION OF INSECT CELL CULTURES

Materials: T-25 flasks in incubation chambers, microscope.
Don't forget to follow the basic aseptic techniques.

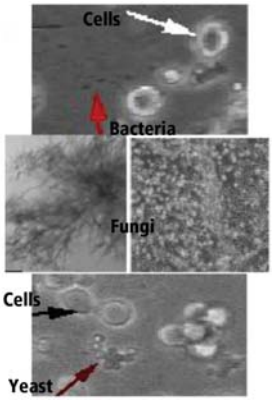
A. Health and Contamination

The most common sources of contamination in cell culture can be:

- Bacteria: medium will appear cloudy and may have a white film on the surface. Under the microscope, the spaces between cells appear granular, with small black dots.
- Fungi: thin filamentous mycelia, can overtake a culture as fuzzy growth (either white or black) that is visible to the naked eye.
- Yeast: round particles that are smaller than insect cells and are usually seen in chains of two or more.

Unhealthy cells show increased granularity, vacuolation, cell shrinkage, cell membrane blebbing and cell fragmentation.

1. Visually examine the insect cell cultures daily under a microscope for signs of contamination and health.
2. Hold flask up against a light source and check if the medium is clear. Since the insect cells grow attached to the surface, the medium in the flask should be clear. A cloudy cell culture medium indicates microbial contamination or the cells are too confluent (too many cells) and need to be subcultured.
3. Examine the cells under a microscope. Look for signs unhealthy cells such as granularity, many vacuoles in the cytoplasm, floating cells, cell membrane blebs, and cell shrinkage. These indicate that the cell medium needs to changed and the cells need to be subcultured.
4. If the cell culture is contaminated, immediately add 1 ml of 10% bleach solution inside the flask and discard the culture.
5. Enter the results of initial examination (status before subculture: appearance of cells, clarity of medium, presence or absence of contamination) of the insect cell culture in the Subculture Data Record.



Student Experimental Procedures

B. Morphology of the Cells

Observation of morphology is the simplest and most direct technique used to identify cells.

Cell morphology can be described as:

- "Fibroblastic" appearance (fibroblastoid), which refers to bipolar or multipolar migratory cells with a length that is twice its width.
- "Epithelial" (or epitheloid) refers to cells that are polygonal, with regular dimensions.
- Round cells (lymphoblastoid) that grow singly or in clumps ("grape-like" clusters).

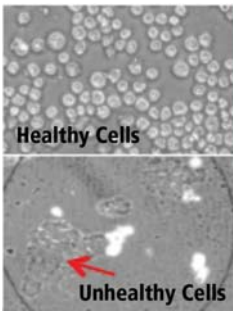
1. Examine the cell morphology of the insect cells daily using the inverted phase contrast microscope.

2. Record your observations in your lab notebook by drawing the shape(s) of your cells. Describe their morphology and characterize them as either fibroblastic, epithelial or lymphoblastoid. Compare the morphology of the cells at the center of a confluent area and at the edges.

3. Determine if the cells are healthy. Unhealthy cells show increased granularity, vacuolation, cell shrinkage, cell membrane blebbing, and cell fragmentation. Record your observations in your lab notebook.

4. If possible, take photomicrographs from the inverted microscope with a digital camera attached. Print out the digital images of your cells and include them in your results.

5. Observe any changes in cell morphology as the cells increase in confluency and go through the cell growth phases (lag, log, and plateau phase). Compare the morphology of your cells at each of the growth phases and record your observations in your lab notebook.



Student Experimental Procedures

C. Phase of Growth Cycle

As cells grow in culture, they go through three distinct phases of growth that can be estimated in terms of confluency and cell density.

- Lag Phase: After subculture or transfer to new flasks, cells enter a lag phase of growth where there is little or no increase in cell number and usually last about 1-2 days. During this time, the cells are "conditioning" the media. Less than 50% of the cell surface is covered by cells (less than 50% confluency) and cell density is low.

- Log Phase: The cell number increases exponentially during this phase, and cell growth will continue as long as there is enough nutrients to sustain the increasing cell number. About 50-80% of the cell surface is covered by cells (50 – 80% confluency) and there is intermediate cell density.

- Plateau Phase: During this phase, the number of cells remains constant (although not necessarily viable). Eventually, the cells will die unless subcultured or fresh media is added. About 90 -100% of the cell surface is covered by cells (90 – 100% confluency) and there is high cell density.

Examine the phase of growth of the insect cells and identify in which phase are the cells and their density. Enter the data in the Subculture data records sheet: status before subculture (phase of growth cycle and cell density).

Student Experimental Procedures

Experiment Procedure

III. MAINTENANCE OF INSECT CELL CULTURES:

One of the most common phenomena in cell culture is when the cells appear unhealthy but they are still less than 50% confluent. One of the main reasons is that the nutrients from the medium have been depleted and toxic metabolites from the cells have been accumulated. The best way to avoid that cells start drying is to feel the cells with new medium.

Materials: T-25 flasks with cells, microscope, 70% Ethanol, Insect Cell medium, pipets or sterile transfer pipets, new T25 flasks. **Don't forget to follow the basic aseptic techniques.**

A. Feeding the Insect Cells

1. Remove the insect cell media from the refrigerator and allow it to equilibrate to room temperature before using.
2. Aspirate 4 ml of the medium in the flask and replace with 4 ml of fresh medium. For optimal growth, leave 1 ml of the old medium in the flask because it contains growth factors that have been secreted by the cells (conditioned medium).
3. Continue to incubate Insect Cells at room temperature in incubation chambers.

B. Subculturing Insect Cells

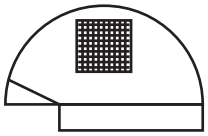
When the cells have reached late log phase of growth and are about 70-80% confluent, subculture the cells into new flasks. The cells continue to grow on the surface of the flask and give rise to a "monolayer" culture, until they reach 100% confluency. At this point, the cells stop dividing because there is no more room to spread. Confluent cells exhibit contact inhibition and become unhealthy and die.

1. Since insect cells grow loosely attached to the surface, rap the bottom of the flask to shake most of the cells loose.
2. Using a 5 ml pipet, pipet the cell suspension up and down several times to detach remaining cells. Pipetting up and down also disperses cells into a single cell suspension (no cell clumps), which is desirable at subculture to ensure an accurate cell count and uniform growth on reseeding.
3. Confirm the detachment of the insect cells under a microscope (either upright or inverted).
4. Transfer 1 ml of the 5 ml of insect cell suspension into a labeled sterile T-25 flask (1 to 5 split ratio) containing 4 ml of Insect Cell Culture medium.
5. Examine the cells under the microscope. Make sure that you have cells in your flask and that your cells are round and clear, not shriveled and dark.
6. Fill in data on split ratio and medium type/serum in the Subculture Data Record.
7. Incubate insect cells at room temperature in a plastic box and leave on the lab bench.
8. After 24 hours, the insect cells should have attached to the surface of the flask. Confirm attachment of cells under the microscope.

Student Experimental Procedures

IV. CELL VIABILITY ASSAYS USING TRYPAN BLUE STAINING

The cell counting chamber commonly known as a "hemocytometer" is a device widely used to count the cells in a specific volume of fluid. In this specific case, the chamber will also be used to differentiate dead from live cells. Trypan Blue stain (which is a vital dye) is excluded by live viable cells whereas dead cells take up the dye and stain blue.



Materials: T25 flask with cells, Trypan Blue stain, Cell Counting Chamber, microcentrifuge tubes, microscope. **Don't forget to follow the basic aseptic techniques.**

A. Counting Live and Dead Cells

1. Obtain a clean plastic cell counting chamber.
2. Retrieve your culture flask from the incubation chamber.
3. Pipet cells up and down three times to disperse cells into a single cell suspension (not cell clumps).
4. Transfer 10 µl of cell suspension into a microcentrifuge tube (or use 1 drop from the small transfer pipet).
5. Add 10 µl (1 drop) of Trypan Blue viability stain to the cells in the tube and incubate for 2 minutes. Trypan Blue is a dye that stains dead cells but is not taken up by live cells.
6. Mix thoroughly by pipetting up and down or tapping bottom to tube (at this point the cells have been diluted 1:2, for a dilution factor of 2).
7. Slowly transfer 20 µl (2 drops) of the Trypan Blue-stained cell suspension to a notch on the bottom left side of one counting area of the cell counting chamber. Allow the area in the chamber to fill by capillary action. Do not over or underfill the chamber!
8. Blot off any surplus fluid and transfer the slide to the microscope.
9. Select 10x objective and focus on grid lines in chamber (cell counting chamber grid). Move the slide so the field you see is the outer grid (the whole grid size is 3 mm x 3 mm and the plate is 0.1 mm). Each small grid (area not divided by any additional lines) is 0.33 mm x 0.33 mm x 0.1 mm.
10. Count all of the cells (living and dead) within the whole grid size. Keep a separate count of viable (clear and bright) and nonviable blue cells. (If it is difficult to count the cells at low power (10x), increase magnification to 40x).

Student Experimental Procedures

A. Counting Live and Dead Cells, continued

Compute cell count/ml and percent cell viability of the cell cultures as follows:

- **Formula Hemocytometer Cells/ml**

Cells/ml = average number of cells per small grid x 90 (multiplication factor) x dilution x 10^3

e.g. Insect Cells diluted 1:5, a total of 50 cells counted in 10 small grids.

Cells/ml=50/10 x 90(factor) x 5 x 10^3 = 2.25 x 10^6

- **Formula Percent Viability**

% Viability = (no. of viable cells / total no. of cells counted) x 100

e.g. Insect Cells observed under the microscope 45 bright cells and 5 blue cells.

45/50 x 100 = 90% Viability

Student Experimental Procedures

B. Plotting Cell Growth Curves

1. Perform a cell count and viability assay as described in the previous section every 24 hours for a week until the cells have reached a plateau phase, where there is no more change in the number of cells/ml of the culture.

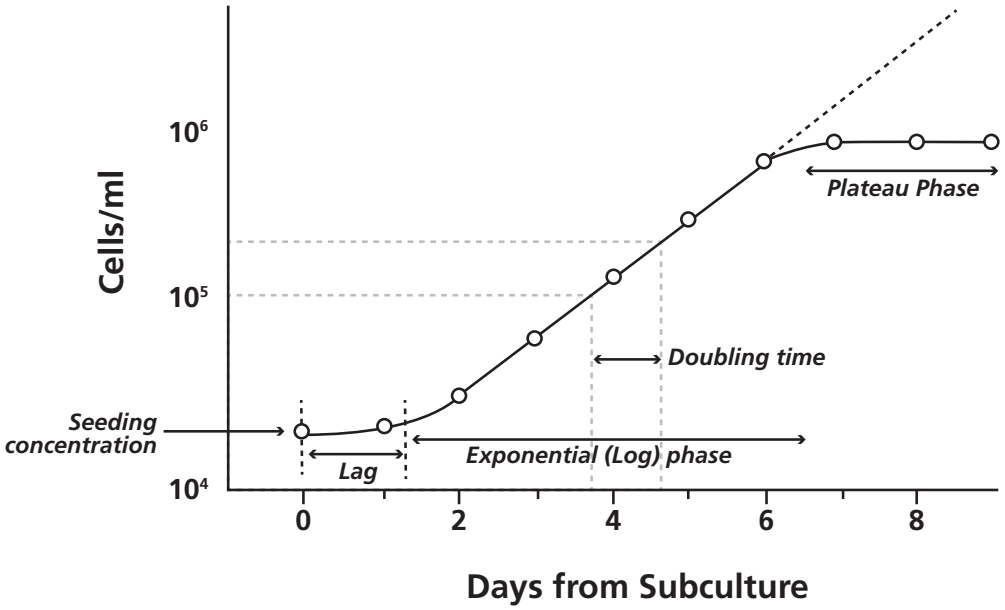
Cells/ml = average number of cells per small grid x 90 (multiplication factor) x dilution x 10^3

Percent Viability= (no. of viable cells / total no. of cells counted) x 100

2. Plot cell concentration (cells/ml) on a log scale against time (in days) of culture.

3. Identify and label the Lag, Log and Plateau growth phases for your cell culture.

4. Select a period of time during the Log Phase and compute the doubling time for your culture. Doubling time is the time required during the Log Phase to exactly double the number of cells/ml. The population-doubling time can be determined by identifying a cell number along the exponential phase of the curve, tracing the curve until that number has doubled, and calculating the time between the two.



Student Experimental Procedures

Experiment Procedure

V. DIFFERENTIAL STAINING ASSAY USING GIEMSA STAIN

Cells are stained with dyes that differentially stain features within the cell making it possible to distinguish finer details. Giemsa stain, a mixture of methylene blue and eosin, allows differential staining of the cell nucleus and the cytoplasm depending on the cell type.

Materials: Flask of cells, 60 mm cell culture plates, Giemsa stain, PBS, Methanol, pipets, pipet aid or transfer pipets. **Don't forget the aseptic techniques.**

A. Growing Cells on a Cell Culture Plate

1. Tap the bottom of the cell culture flask to shake most of the cells loose and pipet the cell suspension up and down several times to detach remaining cells.
2. Transfer 1 ml of insect cell culture from your flask into a small cell culture plate (60 mm) and add 2 ml of fresh Insect Cell culture medium and label plate (for Giemsa staining).
3. Incubate the plate of insect cells for 24 hours in the incubation chambers.

B. Observing Stained Cells Under the Microscope

1. Select a plate with 24 hours old growth. The cells should be attached to the plate. For this procedure, there is no need to maintain aseptic technique.
2. Pour the culture medium out of the plate into the sink and rinse the cells with 5 ml of PBS. Pour off PBS into sink.
3. Fix the cells: Add 2 ml of methanol to cover the cell layer. Fix the cells for 10 minutes at room temperature. Cover the plate to prevent evaporation. Pour out the methanol and air dry cells.
4. Stain the Cells: Add 1 ml of Giemsa stain to the plate to cover cells. Leave Giemsa stain on for 30 seconds and then pour it off.
5. Wash the cells: Add 5 ml of PBS to cover cells for 5 min; pour off PBS and rinse cells with 10 ml of tap water.
6. Examine the morphology of the cells while still wet, using a bright field microscope. Note the differential staining of the nucleus and the cytoplasm. Take photomicrographs if possible.
7. Store dry and re-wet to examine. Record your observations in your lab notebook.

Study Questions

For Questions 1-2: Examine the following microscope images of different cells.

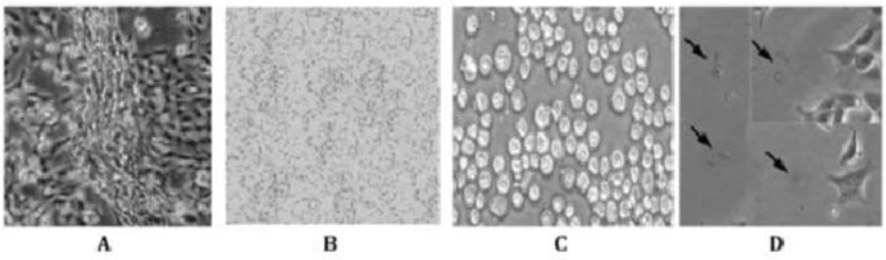


Figure 1

1. From the microscope images in Figure 1, above, identify insect cells.
2. From the microscope images in Figure 1, above, identify bacterial cells.

For Questions 3 - 4: Examine the following typical cell growth curve.

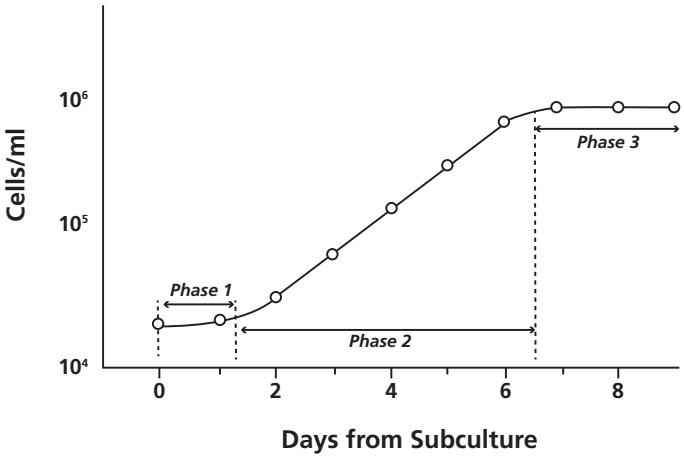


Figure 2

3. In Figure 2, above, which phase represents the lag phase of cell growth?
4. In Figure 2, above, which phase represents the log phase of cell growth?
5. Based on Figure 2, at which phase of cell growth is it best to subculture cells?
6. Based on Figure 2, at which phase of cell growth is it best to feed cells?

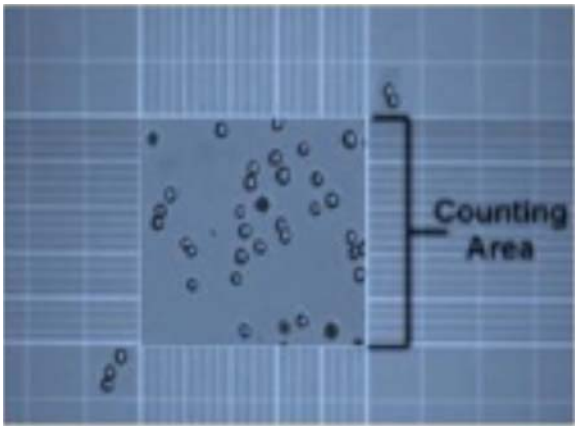
continued

Study Questions, continued

7. What is the optimal temperature for maintaining insect cell culture?

8. If your cell culture became milky and cloudy during an experiment, what has occurred and how was it caused?

9. What is the percent cell viability of the cell culture below? Include the counts for live and total cells.



Instructor's Guide

Notes to the Instructor & Pre-Lab Preparations

IMPORTANT - READ ME!!

Cell Culture experiments contain antibiotics which are used to keep cultures free of contamination. Students who have allergies to antibiotics such as PENICILLIN or STREPTOMYCIN, should not participate in this experiment.

ORGANIZATION AND IMPLEMENTING THE EXPERIMENT

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students.

Prior to commencing this experiment, carefully check that you have all the necessary experiment components and required equipment. Check the lists of Components and Requirements on pages 3 and 4 to ensure that you have a complete inventory to perform the experiment.

The guidelines that are presented in this manual are based on six laboratory groups. The following are implementation guidelines, which can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are available at the EDVOTEK web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call 1-800-EDVOTEK for help from our knowledgeable technical staff.

APPROXIMATE TIME REQUIREMENTS FOR PRE-LAB AND EXPERIMENTAL PROCEDURES

PreLab Preparations (Instructor)

- Recover the cells (one week)
- Prepare the reagents for all the experiments (one hour).

Lab Experiments (Students)

Do all the experiments suggested in the manual (two weeks) divided as follows:

- Learn to perform the basic aseptic techniques and build the incubation containers (one hour)
- Feed/subculture the cells and observe them (one day experiment)
- Giemsa staining (two day experiment)
- Trypan Blue Staining (one day experiment)
- Generation of growth curve (once a day for up to 10 consecutive days)

Pre-Lab Preparations

Before starting any lab experiment or reagent preparation, don't forget to follow the basic aseptic techniques mentioned on page 8.

A. ALIQUOT THE MEDIA

Aseptically aliquot 20 ml of Insect Cell media into six 50 ml tubes. Each group should have its own tube of media to reduce the chance of contamination. Remember to use sterile pipets or transfer pipets.

B. PREPARATION OF INCUBATION CHAMBER

Prepare a large plastic container covered with aluminum foil or cardboard box with a cover. NOTE: EDVOTEK kit box will also work to grow cultures.

The whole group can share a single large container or each student can create his own incubation container. NOTE: An empty autopipette tip box would make a good incubator .

C. INITIATION OF INSECT CELL CULTURE

Provide enough media and flasks to initially inoculate and feed the cells 6 times for 6 groups (Use 2 ml of fresh media each time). One OptiCell culture chamber of cells is provided for the class. (The cells can be seen attached to the clear sides of the OptiCell culture chamber under the microscope.) The kit contains calibrated transfer pipets which can be used for each experiment if sterile disposable pipets, pipet pumps or micropipets are unavailable. The cells will require some time to recover from the shipping and handling.

1. Pre-warm the insect cell culture medium to room temperature.
2. Insect cells grow loosely attached to the surface. Tap the side of the OptiCell culture chamber to release the cells from the sides.
3. Confirm the detachment of the insect cells under a microscope.
4. Wipe the green ports of the OptiCell chamber with 70% Ethanol. Inject 4 ml air into the chamber and remove 4 ml of cell suspension using a 10 ml syringe.
5. Transfer the insect cell suspension into a labeled, sterile T25 flask.
6. Add 2 ml of fresh Insect Cell medium into the T25 flask.
7. Incubate T25 flasks in the incubation chambers.
8. After 24 hours, the insect cells should have attached to the surface of the flask. Confirm attachment of cells under the microscope.

Pre-Lab Preparations

9. Once the cells begin to grow, they can be split into six T25 flasks for the students. Tap the side of the T25 flask and pipet up and down the cells to release the cells from the sides. Take one ml of the suspended cells and add to each new T25 flask containing 4 ml of fresh Insect Cell medium. (If the students want to split the cells, they should follow this procedure). Final volume should be 5 ml.

10. **At this moment, when the cells appear to have stabilized and are growing well, start cell culture experiments with the students.**

D. GIEMSA STAINING OF THE CELLS

Enough supplies and reagents are provided to stain 6 plates of cells.

1. Have the students split cells into culture dishes 24 hours before staining (allow time for cells to attach).

2. Aliquot 10 ml PBS solution into 15 ml tubes and 1 ml Giemsa Stain into 1.5 ml snap cap tubes for each group (6 total).

3. Stained cells can be observed using either an inverted or standard microscope (see note below about how to visualize cells with a standard microscope).

E. PREPARATION OF REAGENTS AND MATERIALS FOR CELL COUNTING AND CELL VIABILITY ASSAYS

To generate a plot of the cell growth curve and identify the phases of cell growth, the students will count the cells once a day for several days (up to 10 days to demonstrate the entire growth curve.)

1. Aliquot individual tubes of 250 µl Trypan Blue for the 6 groups. Each group also receives one counting chamber with 10 wells.

2. Additional counts the following week will complete the plotted curve and illustrate the different phases of growth.

HAVE READY A SIMPLE COMPOUND MICROSCOPE

Most of the experiments will require a simple compound microscope to observe the cells. The cells can be viewed using a standard compound microscope by inverting the flask and placing on the microscope stage. The majority of cells will still be attached to the bottom of the flask and can be visualized. Before inverting the flask, ensure the cap is tightly attached. Cells on tissue culture dishes can also be observed before and after staining by inverting the plate and observing the cells through the bottom of the dish. Ensure the plate is empty of all liquid before inverting. Any spills of cells or media require prompt decontamination with bleach or 70% Ethanol.

Please refer to the kit insert for the Answers to Study Questions

Data Subculture Record

Date ..... Time ..... Operator .....

Cell line	Designation				
	Generation or pass no.				
Status before subculture	Phase of growth cycle				
	Appearance of cells				
	Density of cells				
	Clarity of medium				
Cell count	Concentration after resuspension (C_i)				
	Volume (V_i)				
	Yield ($Y = C_i \times V_i$)				
	Yield per flask				
Seeding	Number (N) & type of vessel (flask, dish, or plate wells)				
	Final concentration (C_F)				
	Volume per flask, dish, or well (V_F)				
	Split ratio ($Y/C_F \times V_F \times N$), or Number of flasks seeded ÷ Number of flasks trypsinized, where the flasks are of same size				
Medium/serum	Type				
	Batch no.				
	Serum type and concentration				
	Batch no.				
	Other additives				
	CO_2 concentration				
	Batch no.				
	Serum type and concentration				
	Batch no.				
	Other additives				

Material Safety Data Sheets

Full-size (8.5 x 11") pdf copy of MSDS is available at www. edvotek.com or by request.

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Safety Statement
This is not an MSDS. According to EU and US regulations, we are not required to supply an MSDS for a product which is not classified as hasardous.

IDENTITY (As Used on Label and List): Fetal Bovine Serum

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **1121 5th Street NW Washington DC 20001**

Emergency Telephone Number: **202-370-1500**

Telephone Number for information: **202-370-1500**

Date Prepared: 08/25/11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous components as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Amber yellow to reddish liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		No data	No data

Extinguishing Media: Water spray, carbon dioxide, dry chem powder or appropriate foam

Special Fire Fighting Procedures: Wear self contained breathing apparatus and protective clothing.

Unusual Fire and Explosion Hazards: No data

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: None

Hazardous Decomposition or Byproducts: Does not produce under normal conditions of storage and use.

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	YEs

Health Hazards (Acute and Chronic): May cause irritation

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
No data	No data	No data	No data

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Inhalation: Remove to fresh air. If breathing difficult, seek emergency help.
Skin: Remove contaminated clothing and shoes. Flush with copious amounts of water for at least 15 minutes.
Eyes: Flush with water for at least 15 min. while separating eyelids with fingers. Call a physician.
Ingestion: If conscious, wash out mouth with water. Call physician.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear protective equipment and clothing. Wash spill site with 10% bleach and ventilate area.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: Non hazardous for transport.

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation				
	Local Exhaust	No	Special	No
	Mechanical (General)	Yes	Other	None

Protective Gloves: YEs — Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Wear protective gloves, safety goggles and lab coat.

Work/Hygienic Practices: Avoid eye and skin contact

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Safety Statement
This is not an MSDS. According to EU and US regulations, we are not required to supply an MSDS for a product which is not classified as hasardous.

IDENTITY (As Used on Label and List): Giemsa Stain

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **1121 5th Street NW Washington DC 20001**

Emergency Telephone Number: **202-370-1500**

Telephone Number for information: **202-370-1500**

Date Prepared: 08/25/11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Giemsa Stain CAS# 51811-82-6 Glycerol CAS# 56-81-5 Methanol CAS# 67-56-1				

Section III - Physical/Chemical Characteristics

Boiling Point	64.5°C	Specific Gravity (H_20 = 1)	0.8
Vapor Pressure (mm Hg.)	No data	Melting Point	-98°C
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Infinite

Appearance and Odor: Clear, dark violet liquid, characteristic alcohol odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
App. 22C, Closed cup		No data	No data

Extinguishing Media: Any means suitable for surrounding fire.

Special Fire Fighting Procedures: Wear Full protective clothing and NIOSH-approved self contained breathing with full facepiece operated in the pressure demand.

Unusual Fire and Explosion Hazards: Flammable liquid. Moderate explosion hazard. Sensitive to static discharge.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	Strong oxidizing agents, heat, sparks, open flame.

Incompatibility: Strong oxidizing agents, heat, sparks, open flame.

Hazardous Decomposition or Byproducts: Acrid and irritating fumes, including toxic formaldehyde and oxides of carbon, when heated to decomposition.

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	YEs

Health Hazards (Acute and Chronic): Primarily toxic by ingestion.

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
No data	No data	No data	No data

Signs and Symptoms of Exposure: May cause skin or eye irritation/burning, dizziness, headache, nausea

Medical Conditions Generally Aggravated by Exposure: Skin dryness, dermatitis. Toxic - avoid contact.

Emergency First Aid Procedures: Inhalation: Remove to fresh air. If breathing difficult, seek emergency help.
Skin: Remove contaminated clothing and shoes. Flush with soap and water for at least 15 minutes.
Eyes: Flush with water for at least 15 min. while separating eyelids with fingers. Call a physician.
Ingestion: Dilute immediately with water or milk. Induce vomiting. Call physician.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Remove all sources of igition. Ventilate area. Contain spill. Absord with inert material (vermiculite, dry sand, etc). Use non sparking tools and equipment.

Waste Disposal Method: Place in a chemical waste container for proper disposal. Observe all local regulations.

Precautions to be Taken in Handling and Storing: Store in a secure, flammable storage area away from all sources of ignition.

Other Precautions: Empty containers can be hazardous since they retain product residues.

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation				
	Local Exhaust	Yes	Special	No
	Mechanical (General)	Yes	Other	None

Protective Gloves: Chem resistant — Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Wear protective gloves, safety goggles and lab coat.

Work/Hygienic Practices: Avoid eye and skin contact

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Safety Statement
This is not an MSDS. According to EU and US regulations, we are not required to supply an MSDS for a product which is not classified as hasardous.

IDENTITY (As Used on Label and List): Penicillin Streptomycin 100X

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **1121 5th Street NW Washington DC 20001**

Emergency Telephone Number: **202-370-1500**

Telephone Number for information: **202-370-1500**

Date Prepared: 08/25/11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Streptomycin sulfate CAS#3810-74-0				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Colorless liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
		No data	No data

Extinguishing Media: Dry chemical, CO2, water spray or regular foam.

Special Fire Fighting Procedures: Wear Full protective clothing and NIOSH-approved self contained breathing with full facepiece operated in the pressure demand..

Unusual Fire and Explosion Hazards:

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None known
	Stable	X	

Incompatibility: None known

Hazardous Decomposition or Byproducts: Carbon oxides, nitrogen oxides, sulfer oxides.

Hazardous Polymerization			Conditions to Avoid
	May Occur		None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? YES

Health Hazards (Acute and Chronic): Primarily toxic by ingestion, inhalation, and skin contact. May cause harm to the unborn child.

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
No data	No data	No data	No data

Signs and Symptoms of Exposure: May cause skin or eye irritation/burning, dizziness, headache, nausea

Medical Conditions Generally Aggravated by Exposure: Toxic - avoid contact.

Emergency First Aid Procedures: Inhalation: Remove to fresh air. If breathing difficult, seek emergency help.
Skin: Remove contaminated clothing and shoes. Flush with soap and water for at least 15 minutes.
Eyes: Flush with water for at least 15 min. while separating eyelids with fingers. Call a physician.
Ingestion: Rinse thoroughly with water and drink plenty of water to dilute. Call physician.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Ventilate area. Absord with inert material. Pick up and transfer to properly labeled containers. Wash spill site after material picku is complete.

Waste Disposal Method: Observe all local regulations.

Precautions to be Taken in Handling and Storing: Keep containers tightly closed in a dry, cool and well-ventilated place.

Other Precautions: Non hazardous for travel.

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation				
	Local Exhaust	No	Special	No
	Mechanical (General)	Yes	Other	None

Protective Gloves: Chem resistant — Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Wear protective gloves, safety goggles and lab coat.

Work/Hygienic Practices: Avoid eye and skin contact

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Safety Statement
This is not an MSDS. According to EU and US regulations, we are not required to supply an MSDS for a product which is not classified as hasardous.

IDENTITY (As Used on Label and List): Cadmium Chloride

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **1121 5th Street NW Washington DC 20001**

Emergency Telephone Number: **202-370-1500**

Telephone Number for information: **202-370-1500**

Date Prepared: 02-15-12

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
CAS# 10108-64-2 CdCl2				

Section III - Physical/Chemical Characteristics

Boiling Point	960°C	Specific Gravity (H_20 = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	568°C
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: No data

Appearance and Odor: clear liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
		No data	No data

Extinguishing Media: Use water spray, dry chem, carbon dioxide or appropriate foam.

Special Fire Fighting Procedures: Wear Full protective clothing and NIOSH-approved self contained breathing with full facepiece operated in the pressure demand.

Unusual Fire and Explosion Hazards: Flammable solid.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Ignition sources, dust generation, heat
	Stable	X	

Incompatibility: Strong oxidizing agents.

Hazardous Decomposition or Byproducts: Nitrogen oxides, carbon monoxide, oxides of sulfur, carbon dioxide, sodium oxide, sodium hydroxide.

Hazardous Polymerization			Conditions to Avoid
	May Occur		None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? YES

Health Hazards (Acute and Chronic): Carcinogen. Target Organ Effect, Highly Toxic by inhalation, Toxic by ingestion, Teratogen, Mutagen

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
Yes	No data	No data	Hazard

Signs and Symptoms of Exposure: Eye: May cause eye irritation

Emergency First Aid Procedures: Inhalation: Remove to fresh air. If breathing difficult, seek medical aid.
Skin: Remove contaminated clothing and shoes. Flush with soap and water for at least 15 minutes.
Eyes: Flush with water for at least 15 min. while separating eyelids with fingers. Call a physician.
Ingestion: Do not induce vomiting. Get medical aid.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Vacuum or sweep up material and place into a suitable disposal container. Avoid generating dusty conditions. Remove sources of ignition. Provide ventilation.

Waste Disposal Method: Place in a chemical waste container for proper disposal. Observe all local regulations.

Precautions to be Taken in Handling and Storing: Store in a secure tightly closed container, flammable storage area away from all sources of ignition. Store in a cool dry place.

Other Precautions: Empty containers can be hazardous since they retain product residues.

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation				
	Local Exhaust	No	Special	No
	Mechanical (General)	No	Other	None

Protective Gloves: Chem resistant — Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Wear protective gloves, safety goggles and lab coat.

Work/Hygienic Practices: Avoid eye and skin contact