

The Biotechnology Education Company ®

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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Eukaryotic Cell Biology Using Insect Cell Culture

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



Eukaryotic Cell Biology Using Insect Cell Culture

Experiment

Storage

Room Temperature 4° C Refrigerator

Room Temperature

Room Temperature

Room Temperature

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

- A Insect Cells (Sf9)
- B Insect Cell Media
- C Trypan Blue Dye
- D Giemsa stain
- E Phosphate Buffered Saline (PBS)
- Cell culture flasks (T-25) 25 cm² (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 *Spodop-tera 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 (Kula-kosky 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 work-sheet.

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.





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.











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.





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.





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.)



Eukaryotic Cell Biology Using Insect Cell Culture



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.
- 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.









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.







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).





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.



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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

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- 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.
- 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).

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Experiment



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 10³

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 10³ = 2.25 x 10⁶

• 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





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 10³

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.



Days from Subculture



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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.
- 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



6. Based on Figure 2, at which phase of cell growth is it best to feed cells?

continued



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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 STREP-TOMYCIN, 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)

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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 Eukaryotic Cell Biology Using Insect Cell Culture



Data Subculture Record

Date

Time

Operator

		The second se		and the second
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)			1
	Volume (V,)		1	
	Yield $(Y = C, \times V,)$	1		1
	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 $(Y/C_r \times V_r \times N)$, or Number of flasks seeded \div Number of flasks trypsinized, where the flasks are of same size			
Medium/serum	Туре			
	Batch no.			
	Serum type and concentration			
	Batch no.			
	Other additives			
	CO ₂ concentration			
	Batch no.			1
	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.

Experiment

					Section V - Reactivity	/ Data					
	This is set a	Safety Statement			Stability	Unstable		Conditio	ons to Avoid		
EDVOTEK.	an MSDS. According to EU and US regulations, not required to supply an MSDS for a product				Stable	Х		None			
which is not classified as hasardous.				Incompatibility None							
IDENTITY (As Used on Label and List)		Note: Blank spaces are	not nermitted. If	anv item is not	Hazardous Decomposition or B Does no	syproducts t produce under i	normal co	ondition	is of storage	and use.	
Fetal Bovine Seru	m	applicable, or no informa be marked to indicate th	ition is available, at.	the space must	Hazardous	May Occur		Conditi	ions to Avoid		
Section I					Polymerization	Will Not Occur	Х	1	None		
Manufacturer's Name		Emergency Telephone Nun	nber 202 27	0 1500	Section VI - Health H	azard Data					
EDVOTEK, Inc.					Route(s) of Entry:	Inhalatio Yes	n?		Skin? Yes		Ingestion? YEs
Address (Number, Street, City, State, Zip Code) 202-370-1500					Health Hazards (Acute and	Chronic) May	cause irr	itation			
1121 5th Street NW		Date Prepared			Carcinogenicity:	NTP2		IAB	C Monogram	nhs?	OSHA Regulation?
Washington DC 20001					No data	No data		No c	data	5113 :	No data
		oignature of rieparer (options			Signs and Symptoms of Exposure May cause skin or eye irritation						
Section II - Hazardous Ingredients/Identify Information					Medical Conditions General	ly Aggravated by	/ Exposu	re			
Hazardous Components (Specific Chamical Identity: Components (Specific Chamical Identity: Components (Continue)					E E 1 1 1 D	Nor	ne reporte	ed			
This product contains no hazardous	components	as defined by the OSHA Haz	ard	/o (Optional)	Skin: Remove contaminated	dures Inhalation d clothing and sh	c Remove loes. Flu:	e to fresi sh with o	n air. If brea copious amo	thing diff: ounts of w	ater for at least 15 minutes.
Communication Standard.					Eyes: Flush with water for a	at least 15 min. v	vhile sepa	arating e	eyelids with	fingers. (Call a physician.
					Section VII - Precauti	sh out mouth wit	th water.	Call phy ling au	nd lise		
Section III - Physical/Chemica	al Charact	eristics			Steps to be Taken in case M	aterial is Releas	ed for Sp	oilled			
Boiling Point		Specific Gravity (H ₀ 0 = 1)		No data	Wear protective equipm	nent and clothing	. Wash s	pill site	with 10% b	leach and	ventilate area.
) (D	No data			NO data	Waste Disposal Method						
vapor Pressure (mm Hg.)	No data	Melting Point		No data	Observe all federal, stat	te, and local regu	ilations.				
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate - 1)		No data	Brooputiess to b. T. t	londline -: 10	rinc				
Solubility in Water	110 data	(Sugradound = 1)		L	Avoid eve and skin con	tariuling and Sto tact.	ning				
Solub	le				,						
Appearance and Odor	r vellow to r	ddish liquid, po odor			Other Precautions Non hazardous for trans	sport.					
Section IV - Physical/Chemic	al Charac	teristics				-r					
Flash Point (Method Used)		Flammable Limits	LEL	UEL	Section VIII - Control	Measures					
No data			No data	No data	Respiratory Protection (Spe	city Type)					
Extinguishing Media Water spi	rav. carbon di	oxide, dry chem powder or at	propriate foar	n	Ventilation	Local Exhaust		No		Special	No
Special Fire Fighting Procedures	<i></i>	, , , , , ,				Mechanical (Ge	neral)	Yes		Other	None
Wear self	contained br	eathing apparatus and protect	ive clothing		Protective Gloves YEs Eye Protection Splash proof goggles						
wear self contained breathing apparatus and protective clothing.					Other Protective Clothing or	Equipment	W		.1		
Unusual Fire and Explosion Hazards					Work/Hygiopia Practices						
No data					Avoid eye and skin contact						
					Section V - Reactivity	v Data					
	This is not a	Safety Statement	US regulations		Section V - Reactivity Stability	Data Unstable		Conditio	ons to Avoid		
EDVOTEK.	This is not a we are no	Safety Statement n MSDS. According to EU and t required to supply an MSDS f	US regulations, or a product		Section V - Reactivity Stability	Unstable Stable	X	Conditio	ons to Avoid g oxidizing a	agents, he	at, sparks, open flame.
EDVOTEK.	This is not a we are no	Safety Statement n MSDS. According to EU and ot required to supply an MSDS f vhich is not classified as hasard	US regulations, or a product ous.		Section V - Reactivity Stability Incompatibility	Data Unstable Stable	X Strong	Conditio Strong oxidizin	ons to Avoid g oxidizing a ng agents, he	agents, he at, sparks	at, sparks, open flame. , open flame.
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IDENTITY (As Used on Label and List) Giemsa Stain Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 1121 5th Street NW Washington DC 20001 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity; Common Name(s)] Giemsa Stain CAS# 5181. Giemsa Stain CAS# 56-81. Methanol CAS# 67-56	This is not a we are no Zip Code) ients/Iden OSHA -82-6 5 1	Safety Statement NSDS. According to EU and trequired to supply an MSDS i which is not classified as hasard Intervention of the state of the state applicable, or no informs be marked to indicate th Emergency Telephone Num Telephone Number for information Date Prepared 08/25/11 Signature of Preparer (optionation) tify Information IPEL ACGIH TLV Record	US regulations, or a product ous. not permitted. If the standards of the standards at the standards 202-37 ation 202-37 ation al) her Limits	any Item is not the space must 0-1500 0-1500 	Section V - Reactivity Stability Incompatibility Hazardous Decomposition or B Acrid and irritating fumes, i Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and C Carcinogenicity: No data Signs and Symptoms of Exp Medical Conditions General Emergency First Aid Procee Skin: Remove contaminated Exercise Jush with water for a Ingestion: Dilute immediat Section VII - Precasa	r Data Unstable Stable yproducts neluding toxic for May Occur Will Not Occur azard Data Inhalatio Yes Chronic) Prim NTP? No data oosure May ly Aggravated by tures Inhalation d clothing and sh at least 15 min. w ely with war Sofe	x Strong ormaldeh x x aarily toxi cause sk / Exposu n dryness : Remov voes. Flu while separ milk. It	Condition Strong yde and Condition No condition No condit	ons to Avoid g oxidizing a g agents, he oxides of ca ions to Avoid None <u>Skin?</u> Yes CG Monograp data e irritation/b titis. Toxic h air. If brea soap and wa soap and wa yeylids with mmiting. Cal	agents, he at, sparks arbon, who phs? - avoid co thing diff ther for at 1 fingers. C Il physicia	at, sparks, open flame. , open flame. en heated to decomposition. Ingestion? Vestion? OSHA Regulation? No data izziness, headache, nausea ntact. icult, seek emergency help. least 15 minutes. Call a physician. in.
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IDENTITY (As Used on Label and List) Giemsa Stain Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 1121 5th Street NW Washington DC 20001 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity, Common Name(s)] Giemsa Stain CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 56.81. Methanol Vapor Pressure (mm Hg.) Vapor Pressure (mm Hg.) Vapor Density (AIR = 1) Solubility in Water Appearance and Odor Clear. Section IV - Physical/Chemice Flash Point (Method Used)	This is not a we are no Zip Code) ients/Iden OSHA -82-6 5 1 al Characi 64.5°C No data No data ie dark violet li al Characi	Safety Statement MSDS. According to EU and trequired to supply an MSDS f which is not classified as hasard Note: Blank spaces are applicable, or no inform be marked to indicate the Emergency Telephone Num Telephone Number for inform Telephone Number for inform Teleph	US regulations, or a product ous. not permitted. If dion is available, at. 202-37 ation 202-37 37 37 37 37 37 37 37 37 37 37 37 37 3	any Item is not the space must 0-1500 0-1500 0-1500 9% (Optional) 0.8 -98°C No data	Section V - Reactivity Stability Incompatibility Hazardous Decomposition or E Acrid and irritating fumes, i Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and the Carcinogenicity: No data Signs and Symptoms of Exp Medical Conditions General Emergency First Aid Procee Skin: Remove contaminate Eyes: Flush with water for a Ingestion: Dilute immediat Section VII - Precauti Steps to be Taken in case M Remove all sources of i (verniculite, dry sand, Waste Disposal Method Place in a chemical was Precautions to be Taken in h Store in a secure, flamm Other Precautions Empty containers can b Section VIII - Control Respiratory Protection (Soe	r Data Unstable Stable yproducts neluding toxic fr May Occur Will Not Occur azard Data Inhalatio NTP? No data osure May Uy Aggravated by Skii tures Inhalation dures Inhalation osure May Uy Aggravated by Skii tures Inhalation ons for Safe tateral is Releas gittion. Ventilate etc). Use non spo ste container for tandling and Sto nable storage are e hazardous since Measures	x Strong yrmaldeh x arily toxi cause sk y Exposu a dryness Removy cause sk y Exposu a dryness Removy a dryness Removy a dryness Removy a dryness Removy a dryness Removy a dryness a drynes	Conditional Strong Stro	ons to Avoid g oxidizing a g agents, he oxides of ca ions to Avoid None <u>Skin?</u> <u>Yes</u> (C Monograp data e irritation/b titits. Toxic h air. If brea soap and wa yeplids with miting. Cal mol U.Se pill. Absord equipment. Observe all sources of ig duct residue:	agents, he at, sparks arbon, who obs? 	at, sparks, open flame. , open flame. en heated to decomposition. Ingestion? OSHA Regulation? No data izziness, headache, nausea ntact. icult, seek emergency help. fail a physician. in. t material ilations.
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IDENTITY (As Used on Label and List) Giemsa Stain Section 1 Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 1121 5th Street NW Washington DC 20001 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity, Common Name(s)] Giemsa Stain CAS# 518.11 Gitycerol CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 518.12 Solubility in Water Appearance and Odor Flash Point (Method Used) App. 220 Extinguishing Media Any mea	This is not a we are n n n n n n n n n n n n n n n n n n n	Safety Statement MSDS. According to EU and trequired to supply an MSDS t which is not classified as hasard Note: Blank spaces are applicable, or no inform be marked to indicate the Emergency Telephone Num Telephone Number for inform Date Prepared 08/25/11 Signature of Preparer (option Titly Information .PEL ACGIH TLV Rec eristics Specific Gravity (Hg0 = 1) Melting Point Evaporation Rate (Butyl Acetate = 1) quid, characteristic alcohol ou teristics Flammable Limits r surrounding fire.	US regulations, or a product ous. not permitted. If that is available, at a valiable, at a valiable, 202-37 202-37 202-37 all) her Limits commended dor dor	any item is not the space must 0-1500 0-1500 (Optional) % (Optional) 0.8 -98°C No data	Section V - Reactivity Stability Incompatibility Hazardous Decomposition or B Acrid and irritating fumes, i Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and the Carcinogenicity: No data Signs and Symptoms of Exp Medical Conditions General Emergency First Aid Procee Kin: Remove contaminate Eyes: Flush with water for a Ingestion: Dilute immediat Section VII - Precauti Steps to be Taken in case M Remove all sources of i (verniculite, dry sand, Waste Disposal Method Place in a chemical was Emergency Forta Land Precautions to be Taken in H Store in a secure, flamm Other Precautions Empty containers can b Section VIII - Control Respiratory Protection (Sper Ventilation	r Data Unstable Stable Stable Stable Stable Stable May Occur Will Not Occur Will Not Occur Prim azard Data Inhalatic Yes Chronic) Prim NTPP No data Skii tures Inhalation I clothing and sho Skii tures Inhalation I clothing and sho Skii tures Inhalation I clothing and Sto anable storage are te chazardous since Measures city Type) Local Exhaust	x Strong marily toxi cause sk v Exposu of drivess Removies rmilk. Ir e Handy cause sk v Exposu of drivess rmilk. Ir e Handy a rava rming to proper di reing to proper di to to to to to to to to to to	Condition Strong oxidizin yde and Condition ic by ing No c in or cycr re c, dermaal c, dermaal sh with a sposal. ror all s tain proc	ons to Avoid g oxidizing a g agents, he oxides of ca ions to Avoid None <u>Xskin?</u> gestion. C Monograp data e irritation/b titits. Toxic - h air. If brea soap and wa veylids with moniting. Cal mol USE pill. Absord equipment. Observe all sources of ig duct residue:	agents, he at, sparks arbon, who ohs? - avoid coo thing diff ter for at ll physicia with iner local regu pation. s. Special Other	at, sparks, open flame. , open flame. en heated to decomposition. Ingestion? OSHA Regulation? No data izziness, headache, nausea ntact. icult, seek emergency help. feast 15 minutes. all a physician. in. t material Jlations. No No No No
IDENTITY (As Used on Label and List) Giemsa Stain Section 1 Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 1121 5th Street NW Washington DC 20001 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity, Common Name(s)] Chemical Identity, Common Name(s)] Chemical Identity, Common Name(s)] Chemical Identity, Common Name(s)] Giemsa Stain CAS# 56.81 Section III - Physical/Chemic Boiling Point Vapor Pressure (mm Hg.) Vapor Density (AIR = 1) Solubility in Water Appearance and Odor Clear, Section IV - Physical/Chemic Flash Point (Method Used) App. 22C Extinguishing Media Any mea	This is not a we are n n n n n n n n n n n n n n n n n n n	Safety Statement MSDS. According to EU and trequired to supply an MSDS 1 which is not classified as hasard Note: Blank spaces are applicable, or no inform be marked to indicate the Emergency Telephone Num Telephone Number for inform. Telephone Number for inform. Telephone Number for inform. Telephone Number for inform. Date Prepared 08/25/11 Signature of Preparer (option: Telephone Number for inform. Date Prepared 08/25/11 Signature of Preparer (option: Date Prepared 08/25/11 Signature of Preparer (option: Signature of Preparer (option: Signature of Preparer (option: Date Prepared 08/25/11 Signature of Prepared Signature of Prepared Signa	US regulations, or a product ous. not permitted. If this is available, at a valuable of the second s	any liam is not the space must 0-1500 0-1500 % (Optional) 0.8 98°C No data	Section V - Reactivity Stability Incompatibility Hazardous Decomposition of B Acrid and irritating fumes, it Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and C Carcinogenicity: No data Signs and Symptoms of Exp Medical Conditions General Emergency First Aid Procee Skin: Remove contaminate Eyes; Flush with water for a Ingestion: Dilute immediat Steps to be Taken in case M Remove all sources of i (vermiculite, dry sand, Waste Disposal Method Place in a chemical was Precautions to be Taken in H Store in a secure, flamm Other Precautions Empty containers can b Section VIII - Control Respiratory Protection (Spe Ventilation	r Data Unstable Stable Stable Stable Stable Stable May Occur Mill Not Occur azard Data Inhalatic Yes Chronic) Prim NTP? No data 1005Ur6 May NTP? No data 1005Ur6 May NTP? No data 1005Ur6 May NTP? No data 1005Ur6 May 10 Aggravated by Siti Ures Inhalation 1 clothing and Sto nable storage are te container for tandling and Sto nable storage are cify Type) Local Exhaust Mechanical (Ge tem resistant	X Strong prmaldch, X X X x x x x x x x x x x x x x x x x	Condition Strong oxidizin yde and Conditizin ic by ing IAR No c in or eye re c, dermaal sh with h sho sh with sh with sh sh sh sh sh sh sh sh sh sh sh sh sh sh sh s	ons to Avoid g oxidizing a g agents, he oxides of ca ions to Avoid None <u>Skin?</u> gestion. IC Monograp data e irritation/b titits. Toxie - h air. If brea soap and wa yellak with miting. Cal nd Use pill. Absord equipment. Observe all sources of ig duct residue: Eye Protec	agents, he at, sparks at, sparksp	at, sparks, open flame. , open flame. en heated to decomposition. Ingestion? VES OSHA Regulation? No data izziness, headache, nausea ntact. icult, seek emergency help. call a physician. in. t material tlations. No No No No No No Splash proof goodes
IDENTITY (As Used on Label and List) Giemsa Stain Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 1121 5th Street NW Washington DC 20001 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity, Common Name(S) Chemical Identity, Common Name(S) Methanol CAS# 57.66 Section III - Physical/Chemic Boiling Point Vapor Pressure (mm Hg.) Vapor Density (AIR = 1) Solubility in Water Appearance and Odor Clear, Section IV - Physical/Chemic Flash Point (Method Used) Flash Point (Method Used) Flash Point (Method Used) Special Fire Fighting Proceduce Special Fire Fighting Proceduce Wear Full Self cont	This is not a we are n n n n n n n n n n n n n n n n n n n	Safety Statement MSDS. According to EU and trequired to supply an MSDS 1 which is not classified as hasard Note: Blank spaces are applicable, or no inform be marked to indicate th Emergency Telephone Num Telephone Number for inform. Telephone Number for inform. Telephone Number for inform. Telephone Number for inform. Date Prepared 08/25/11 Signature of Preparer (option. Telephone Number for inform. Date Prepared 08/25/11 Signature of Preparer (option. Telephone Number for inform. Date Prepared 08/25/11 Signature of Preparer (option. Date Prepared 08/25/11 Signature of Prepared Signature of Prepared Sign	US regulations, or a product ous. not permitted. If thion is available, at. 202-37 ation 202-37	any liam is not the space must 0-1500 0-1500 % (Optional) % (Optional) 0.8 98°C No data	Section V - Reactivity Stability Incompatibility Hazardous Decomposition of B Acrid and irritating fumes, i Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and H Carcinogenicity: No data Signs and Symptoms of Exp Medical Conditions General Emergency First Aid Procee Skin: Remove contaminate Eyes: Flush with water for a Ingestion: Dilute immediat Steps to be Taken in case M Remove all sources of (vermiculite, dry sand, Waste Disposal Method Place in a chemical was Precautions to be Taken in H Store in a secure, flamm Other Precautions Empty containers can b Section VIII - Control Respiratory Protection (Spe Ventilation Protective Gloves Cth	r Data Unstable Stable Stable Stable Stable Stable Stable Stable Stable Stable Stable May Occur Will Not Occur azard Data Inhalatic NTP? No data Sosure May Nump? No data Sosure May Nump? No data Stable S	x Strong yrmaldch x X x on? arily toxi cause sk y Exposu a dryness r milk. Ir b Hand ead for Scp r milk. Ir b Hand r for Scp r milk a rea. Cr arking to r ming a away fr r ming r e they re inneral)	Condition Strong oxidizin yde and Conditi No c in or eye re c, dermala sh with a sho sh in or eye re c, dermala sh with a sho sh in or eye re c, dermala sh with a sho sh in or eye re c, dermala sh with a sposal.	ons to Avoid g oxidizing a g agents, he oxides of ca ions to Avoid None Skin? gestion. IC Monograp data e irritation/b titis. Toxic - th air. If bre- asoap and wa yelids with bomiting. Cal nd USE pill. Absord equipment. Observe all sources of ig duct residue: Eye Protec	agents, he at, sparks at, sparksp	at, sparks, open flame. , open flame. en heated to decomposition. Ingestion? VES OSHA Regulation? No data izziness, headache, nausea nitact. icult, seek emergency help. Call a physician. in. t material ilations. No No Splash proof goggles
IDENTITY (As Used on Label and List) Giemsa Stain Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 1121 5th Street NW Washington DC 20001 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity, Common Name(s)] Giemsa Stain CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 56.81. Methanol CAS# 57.56 Section III - Physical/Chemic Boiling Point Vapor Pressure (mm Hg.) Vapor Density (AIR = 1) Solubility in Water Flash Point (Method Used) Flash Point (Method Used) Flash Point (Method Used) Special Fire Fighting Procedute Self conta	This is not a we are n n we are n n ZIp Code) ients/Iden OSHA -82-6 5 -1 al Charact 64.5°C No data No data e dark violet li al Characc 2, Closed cup ns suitable fo 1 protective cl inined breathin	Safety Statement MSDS. According to EU and trequired to supply an MSDS t which is not classified as hasard Note: Blank spaces are applicable or no inform be marked to indicate the Emergency Telephone Num Telephone Number for inform Date Prepared 08/25/11 Signature of Preparer (option Titly Information .PEL ACGIH TLV Rec eristics Specific Gravity (Hg0 = 1) Melting Point Evaporation Rate (Butyl Acetate = 1) quid, characteristic alcohol or teristics Flammable Limits r surrounding fire. othing and NIOSH-approved g with full facepiece operate	US regulations, or a product ous. not permitted. If those available, at available, at available, 202-37 202-37 202-37 all) her Limits commended dor let LEL No data	ary tem is not the space must 0-1500 0-1500 0-1500 (Optional) % (Optional) % (Optional) 0.8 -98°C No data UEL No data re demand.	Section V - Reactivity Stability Incompatibility Hazardous Decomposition or E Acrid and irritating fumes, i Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and the Carcinogenicity: No data Signs and Symptoms of Exp Medical Conditions General Emergency First Aid Procee Skin: Remove contaminate Eyes: Flush with water for a Ingestion: Dilute immediat Section VII - Precauti Steps to be Taken in case M Remove all sources of i (verniculite, dry sand, Waste Disposal Method Place in a chemical was Empty containers can b Section VIII - Control Respiratory Protection (Spe Ventilation Protective Gloves Ch Other Protective Clothing or	r Data Unstable Stable Stable Stable Stable Stable May Occur Will Not Occur Prim azard Data Inhalatic Yes Chronic) Prim NTPP No data Sosure May Uy Aggravated by Skii tures Inhalation I clothing and Sto anable storage are te cantainer for tandling and Sto anable storage are te hazardous since Meesanical (Ge tem resistant Equipment	x Strong rrmaldeh rrmaldeh x X x x x x x x x x x x x x x x x x	Conditional Strong Stro	ons to Avoid g oxidizing a g agents, he oxides of ca ions to Avoid None <u>X</u> Skin? gestion. C Monograp data e irritation/b titits. Toxic - h air. If brea soap and wa yeylids with moniting. Cal mol USE pill. Absord equipment. Observe all sources of ig duct residue: Eye Protecc gloves, safet	agents, he at, sparks arbon, whe ohs? - avoid coo thing diff ter for at II physicia with iner local regu pation. s. Special Other ttion	at, sparks, open flame. , open flame. en heated to decomposition. Ingestion? OSHA Regulation? No data izziness, headache, nausea ntact. icult, seek emergency help. feast 15 minutes. all a physician. in. t material ilations. No No No No No No Splash proof goggles and lab coat.

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



						Section V - Reactivi	ty Data					
	Safety Statement			Stability	Unstable		Conditions to Av	/oid		_		
EDVOTEK.	n MSDS. ot require	 According to EU an ed to supply an MSD; 	d US regulations, S for a product			Stable	Х	None known				
which is not classified as hasardous.					Incompatibility		None	known				
IDENTITY (As Used on Label and List)			Note: Blank spaces a	are not permitted. If	any item is not	Hazardous Decomposition of Carbon oxides, nitrogen o	r Byproducts xides, sulfer oxide	es.				
Penicillin Streptor	nycin 100X		applicable, or no infor be marked to indicate	mation is available, that.	the space must	Hazardous	May Occur		Conditions to A	void		
Section I	-					Polymerization	Will Not Occur	Х	None			
Manufacturer's Name Emergency Telephone Number					Section VI - Health	Hazard Data		•			_	
EDVOTEK Inc				0-1500	Route(s) of Entry:	Inhalati	on?	Skin	?	Ingestion?		
Address (Number, Street, City, State, Zip Code)			Telephone Number for information 202-370-1500			Yes Yes YEs Health Hazards (Acute and Chronic)						_
1121 5th Street NW		Date P	repared			Primarily toxic by ing	gestion, inhalation	, and ski	n contact. May	cause harm to	the unborn child.	
Washington DC 20001			08/25/11			No data	NIP? No data		No data	graphs?	OSHA Regulation? No data	
		Signati	ure of Preparer (optic	onal)		Signs and Symptoms of E	xposure May	y cause s	kin or eye irritati	on/burning,	dizziness, headache, nause	a
Section II - Hazardous Ingred	lients/Iden	tify In	formation			Medical Conditions Gener	rally Aggravated b	y Exposi	ure			-
Hazardous Components [Specific	0011			Other Limits	% (Ontional)	-	To	xic - avo	id contact.			_
Streptomycin sulfate CAS#3810-7	14-0	APEL	ACGIH ILV H	recommended	% (Optional)	Emergency First Aid Proc Skin: Remove contaminat	edures Inhalation ted clothing and sl	n: Remov hoes. Fli	ve to fresh air. If ish with soap an	breathing dif d water for at	ficult, seek emergency hel t least 15 minutes.	lp.
						Eyes: Flush with water fo	r at least 15 min.	while sep	parating eyelids v	with fingers.	Call a physician.	
						Ingestion: Rinse thoroug	hly with water and	l drink p	lenty of water to	dilute. Call	physician.	
Section III - Physical/Chemic	al Charact	teristic	cs			Section VII - Precau	tions for Saf	e Hand	lling and Us	е		_
						Steps to be Taken in case Ventilate area Absor	Material is Released with inert mater	sed for S	pilled	to properly l	abeled containers	
Boiling Point	No data	Spec	cific Gravity (H ₂ 0 =	1)	No data	Wash spill site after material picku is complete.						
Vapor Pressure (mm Hg.)	No data	Melti	ing Point		No data	Waste Disposal Method Observe all local regulations.						
Vapor Density (AIB = 1)	110 uuu	Evap	poration Rate		N. L.	·						
	No data	(Buty	yl Acetate = 1)		No data	Precautions to be Taken in Keep containers tight	Handling and Sto	oring	well-ventilated	nlace		
Solubility in Water Solub	le					Reep containers tight	ry closed in a dry,	coor and	r went-ventilated	place.		
Appearance and Odor Color	less liquid, no	o odor				Other Precautions Non hazardous for tra	avel.					
Section IV - Physical/Chemic	al Charac	teristi	cs									_
Flash Point (Method Used)		Flam	mable Limits	LEL	UEL	Section VIII - Contro	oi measures					
				No data	No data	Respiratory Protection (S	pecity type)					
Extinguishing Media Dry cher	nical CO2 w	ater spra	av or regular foam			Ventilation	Local Exhaust		No	Special	No	
		uter spre	iy or regular routin				Mechanical (Ge	eneral)	Yes	Other	None	
Special Fire Fighting Procedures Wear Ful self conta	ll protective c ained breathir	lothing ang with f	and NIOSH-approv full facepiece opera	ed ted in the pressu	re demand	Protective Gloves	Chem resistant		Eye Pi	rotection	Splash proof goggles	
Unusual Fire and Explosion Hazards						Other Protective Clothing	or Equipment	Wear pr	otective gloves,	safety goggle	es and lab coat.	
						Work/Hygienic Practices	Avoid ey	ye and sk	in contact			
						-						
						Section V - Reactivi	ty Data					-
		Sat	fety Statemen	t		Stability Unstable Conditions to Av			Avoid			
EDVØTEK.	This is not a we are not	n MSDS. ot require	According to EU an to supply an MSDS	d US regulations, S for a product			Stable	Х	Ignition sources, dust generation, heat			
		which is n	not classified as hasa	irdous.		Incompatibility		Strong	oxidizing agent	s.		_
						Hazardous Decomposition or	Byproducts	0.10	1			_
IDENTITY (As Used on Label and List)			Note: Blank spaces a applicable, or no infor	re not permitted. If mation is available,	any item is not the space must	Nitrogen oxides, carbon m	ionoxide, oxides c	or sulfur,	carbon dioxide,	socium oxid	e, sodium hydroxide.	
Cadmium Chloride be marked to indicate that.					Polymerization May Occur Conditions to Avoid				lvoid			

					Hazardous Decomposition	or Byproducts	and a deside and			
IDENTITY (As Used on Label and List) Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must					Nitrogen oxides, carbon	monoxide, oxides of sulfur,	Cardon dioxide, sodi	im oxide, sodium nydroxide.		
Cadmium Chlorid	le	be marked to indicate that.			Polymerization	May Occur	Conditions to Avoid			
Section I					топулистидатон	Will Not Occur A	None			
Manufacturer's Name	E	Emergency Telephone Nu	umber 202-37	70-1500	Section VI - Health	n Hazard Data				
EDVOTEK, Inc.		alanhana Number fer infer	202-31	0-1300	Route(s) of Entry:	Inhalation? Yes	Skin? Yes	Ingestion?		
Address (Number, Street, City, State,	Zip Code)	elephone Number for Infon	202-37	0-1500	Health Hazards (Acute a	nd Chronic)				
1121 Eth Streat NW	7	Date Prepared			Carcinogen. Target Organ Effect, Highly Toxic by inhalation, Toxic by ingestion, Teratogen, Mutagen					
Washington DC 20001		02-15-12			Carcinogenicity:	NTP? No data	No data	INS? OSHA Regulation?		
Washington DC 20001	S	Signature of Preparer (optio	onal)		Signs and Symptoms of	Exposure	NO Uala	Hazaiu		
							+:			
Section II - Hazardous Ingree	dients/Identif	y Information			Eye: May Ca	ause eye imita	uon			
Hazardous Components [Specific Chemical Identity: Common Name(s)			Other Limits	% (Ontional)	Emorgonov Eirot Aid Bro	anduron Inhalation Domo		deine difficult and and direct and		
CAS# 10108-64-2			ooonninondod	/= (= = =====	Skin: Remove contamin	ated clothing and shoes. Flu	ish with soap and wa	ter for at least 15 minutes.		
CdCl2					Eyes: Flush with water f	for at least 15 min. while sep	arating eyelids with	fingers. Call a physician.		
					Ingestion: Do not induc	e vomiting. Get medical aid	1.			
O a atticing III. Dhousing al/Oh annia					Section VII - Preca	utions for Safe Hand	lling and Use			
Section III - Physical/Chemic		istics			Steps to be Taken in cas	e Material is Released for S	pilled			
Boiling Point	1 1	Specific Gravity (H ₂ 0 =	1)	N. L.	Vacuum or sweep u	p material and place into a s	uitable disposal conta	iner. Avoid generating dusty		
	960°C	2		No data	conditions. Remove	e sources of ignition. Provid	le ventilation.			
Vapor Pressure (mm Hg.)	No data	Melting Point		568°C	Place in a chemical waste container for proper disposal. Observe all local regulations.					
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)		No data	Precautions to be Taken	in Handling and Storing				
Solubility in Water					Store in a secure tig	htly closed container, flamm	able storage area awa	ay from all sources of ignition.		
No d	ata				Store in a cool dry p	blace.				
Appearance and Odor clear	liquid, no odor				Other Precautions Empty containers ca	an be hazardous since they r	etain product residues			
Section IV - Physical/Chemi	cal Character	ristics			Section VIII - Cont	rol Measures				
Flash Point (Method Used)		Flammable Limits	LEL No data	UEL No data	Respiratory Protection (Specify Type)				
Extinguishing Media			ivo uata	NO Uata		Lucie Luci	N	Special N		
Use wat	er spray, dry cher	n, carbon dioxide or app	ropriate foam.		Ventilation	Local Exhaust	No	Outras None		
Special Fire Fighting Procedures -						Mechanical (General)	110	Other None		
Wear Fu	ill protective cloth	hing and NIOSH-approv	ed		Protective Gloves	Chem resistant	Eye Protec	tion Splash proof goggles		
self con	tamed breatning v	with full facepiece operat	ieu in ine pressu	ne demand.	Other Brotestive Clathing	a or Equipmont				
Unusual Fire and Explosion Hazards					Other Protective Clothing	y or Equipment Wear pr	otective gloves, safet	y goggles and lab coat.		
Flamma	ble solid.				Work/Hygienic Practices	Avoid eye and sk	in contact			