Edvo-Kit # 990

Edvo-Kit #990

Morphology of Cancer Cells

Experiment Objective:

In this lab, students will explore the morphology of normal and cancerous cells by staining pre-fixed cells with methylene blue and eosin. The cell morphology will then be examined using light microscopy. After comparing the two cell types, students will identify the cancerous cells.

See page 3 for storage instructions.

Version 990.240206

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Table of Contents

Safety Data Sheets can be found on our website: www.edvotek.com/Safety-Data-Sheets

Experiment Components

Component	Check (✓)
• Ready-to-Stain slides containing normal and cancer cells	❑
• Rehydration buffer	❑
• Eosin stain solution	❑
• Methylene Blue stain solution	❑
Supplies	
• Transfer pipets	❑
• Pre-stained control slide	❑

Storage:
Room Temperature

This experiment is designed for 6 groups.

Requirements *(NOT included with this experiment)*

- Microscopes (100X total magnification recommended)
- Forceps
- Distilled water
- Gloves
- Paper Towels or Kimwipes
- Beakers (250 mL or larger recommended)
- Timers
- Slide coverslips (optional)
- Mounting medium (optional)

Background Information

UNDERSTANDING THE GENETIC AND MOLECULAR BASIS FOR CANCER

Cancer is one of the leading causes of death in the United States of America, contributing to almost one in every four deaths. Almost everyone has been touched by cancer, either through personal experience or through the impact of the disease on someone they know. Fortunately, progress continues to be made and the life expectancies for many cancer patients have steadily increased over the past few decades. Researchers are continually improving our understanding of the molecular and genetic mechanisms behind the development of cancer. These new discoveries, combined with traditional microscopic observations, have increased our understanding of the distinctions between normal and cancer cells.

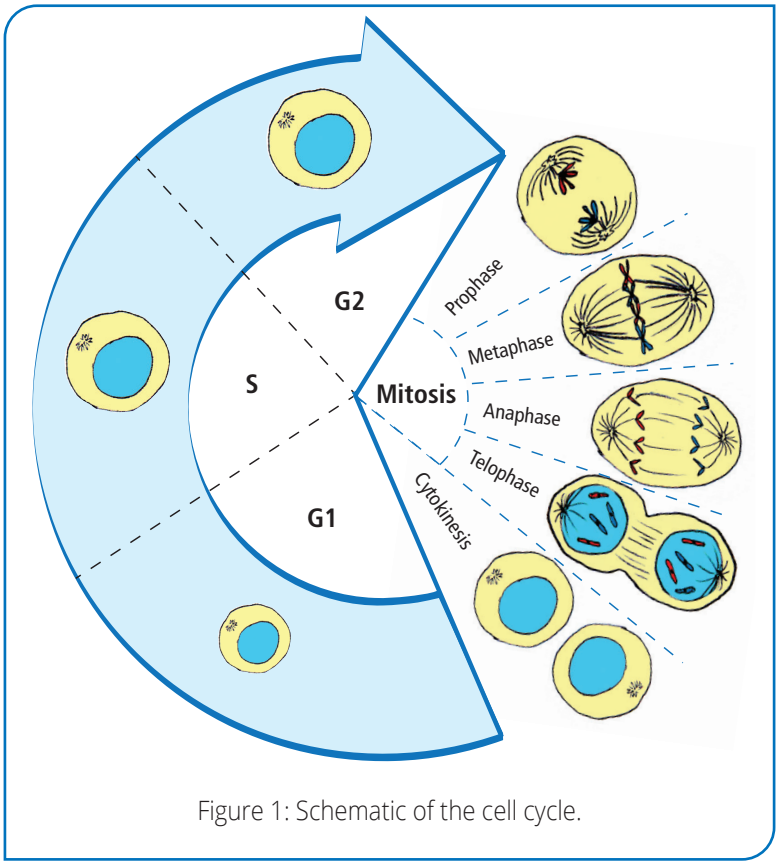


Figure 1: Schematic of the cell cycle.

THE CELL CYCLE DURING CANCER PROGRESSION

The cell cycle is a series of biochemical signaling pathways that drive cells through growth and proliferation. This process, involving the duplication of DNA during mitosis and the division of the cell through cytokinesis, results in two identical daughter cells. Normal cell division has four discrete phases known as M, G1, S, and G2. In between each of these phases are tightly controlled molecular roadblocks known as checkpoints that regulate passage through cell division (Figure 1). This series of coordinated events controls the proliferation, development, and maintenance of cells in an organism.

The growth and death of eukaryotic cells is strictly controlled. Cells use extracellular signals to regulate the rate and location of division. Additionally, if severe DNA damage occurs, normal cells can trigger apoptosis, or regulated cell death (Figure 2A). In contrast, mutations can provide a mechanism that allows cells to bypass the cell cycle checkpoints and inhibit apoptosis, leading to cancer. In cancerous cells mitosis can take place continuously, generating an over-abundance of cells that can form tumors and spread throughout the body.

TUMOR SUPPRESSOR PROTEINS AND ONCOGENES

There are many theories explaining how a healthy cell can become cancerous. In general, most cancer cells seem to result from mutations in genes controlling cellular growth. These mutations then lead to either the gain or loss of important protein functions, the destabilization of the cell, and eventually to the development of cancer.

Proto-oncogenes are a group of genes which code for proteins that regulate cell growth and division. During the early steps in cancer progression a mutation, or combination of multiple mutations, can lead to the conversion of a proto-oncogene into an **oncogene**. Once converted, oncogenes become more active and repeatedly signal for the cell to divide. Many oncogenes are dominant, meaning that a mutation is needed in only one copy of the gene in diploid cells. Cells with oncogenic mutations are unable to control cell proliferation, one of the primary promoters of cancer formation (Figure 2B). For example, the *Myc* oncogene is mutated in many different cancers, including breast, lung, colon, and stomach. The activated *Myc* oncogene can control up to 15% of the genes in a cell, making it a primary driver of cell proliferation.

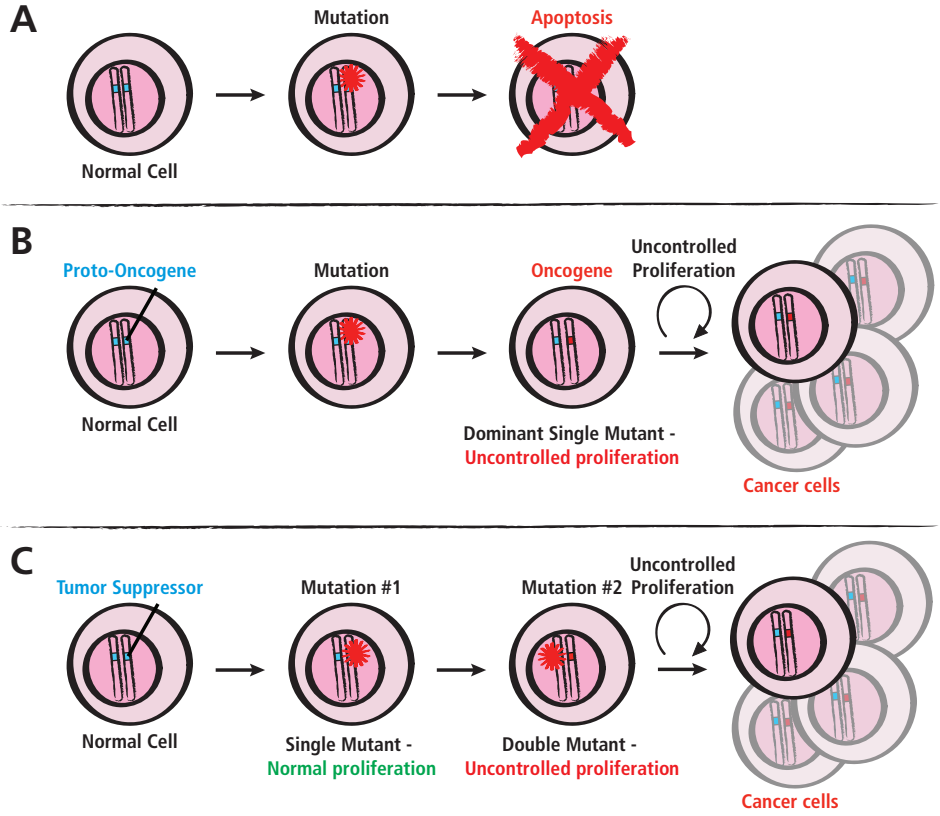


Figure 2: Oncogenes and tumor suppressors regulate cancer progression.

In contrast to oncogenes, **tumor suppressor** proteins are able to inhibit cell growth and prevent tumor formation. Mutations in tumor suppressor genes can inactivate or destroy the corresponding proteins. In this way, a mutation in a tumor suppressor gene removes one of the barriers preventing uncontrolled cell proliferation and tumor formation (Figure 2C). It is important to remember that normal human cells are diploid, containing two copies of every gene. Because of this, a single tumor suppressor mutation can often be compensated for by the remaining normal gene on the duplicate chromosome. In these cases both copies of the gene must be mutated in cancerous cells.

One example of a tumor suppressor protein is p53, encoded by the *TBP53* gene. P53 is an important protein in tumor suppression, regulating DNA damage repair, arresting cell growth, and initiating apoptosis in unhealthy cells. Homozygous loss of p53, where mutations have arisen in both copies of the gene, is found in 65% of colon cancers, 30-50% of breast cancers, and 50% of lung cancers. Due to the prevalence of p53 mutations, and the importance of the gene in driving cancer progression, it has become one of the most well studied cancer genes.

Mutations in oncogenes and tumor suppressors are often the driving force behind cancer formation, and it is rare for cancer cells to contain only a single mutation. In fact, the initial mutations in pre-cancerous cells can lead to additional genetic instability, leading to further mutations in that cell and its daughter cells. Cancer cells will often contain one or more oncogenes combined with the inactivation of an important tumor suppressor.

THE CHARACTERISTICS OF CANCER CELLS

While uncontrolled cell growth is the defining characteristic of cancer, there are several additional qualities that distinguish cancer cells from their normal counterparts. For example, normal cells are difficult for scientists to grow in the laboratory. These cells are generally very sensitive to the cell culture conditions and demand specialized treatments and media. Additionally, normal cells will only divide a few times before arresting. In contrast, tumor cells are often much easier to culture, readily proliferate in the laboratory and divide indefinitely.

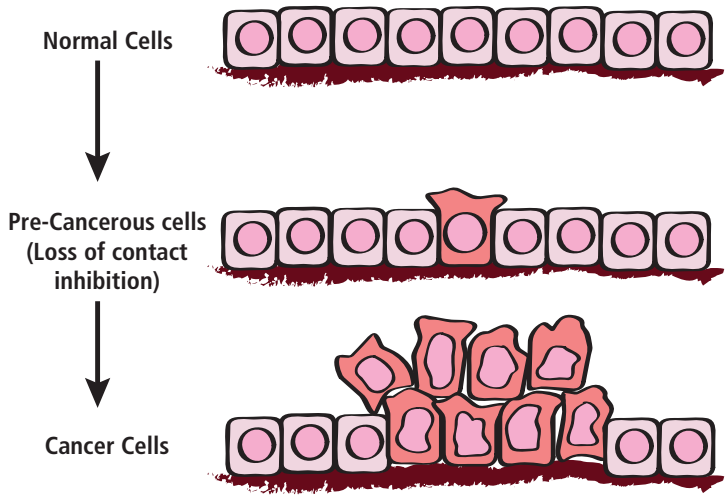


Figure 3: The growth characteristics of normal and cancer cells.

Another important distinction between normal and cancer cells concerns a mechanism known as contact inhibition. Normal cells will divide until they are in contact with the neighboring cells, at which point they stop growing. Thus, contact inhibition results in a sheet of cells just one layer thick, referred to as a monolayer. Cancer cells typically lose contact inhibition, causing them to pile up and form tumors (Figure 3). In addition, cancer cells often become less adherent, both to other cells and to the extracellular matrix. This occurs as a result of changes in cell-surface glycoproteins, altering a cell's ability to form proper connections.

Together, these characteristics produce the most devastating effects of cancer. Uncontrolled growth results in painful and dangerous tumors, displacing normal cells and destroying surrounding tissues. Changes in adherence and contact inhibition allow cancer cells to migrate away from the original tumor and grow in other parts of the body, known as metastasis. In fact, the spread of cancer throughout the body is responsible for most of the suffering and death seen in cancer patients.

CANCER CELL MORPHOLOGY AND ANALYSIS

Along with genomic alterations and accelerated growth, physical characteristics can be used to classify cancer cells. The nuclear structure of cancer cells undergoes changes that result in a large, irregularly shaped nucleus, and modifications to the chromosomes. These morphological characteristics have been considered the "gold-standard" for diagnosing cancer.

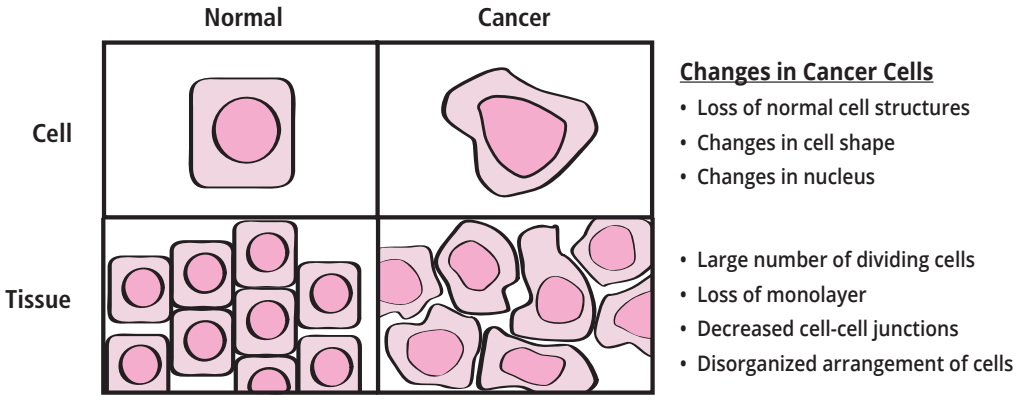


Figure 4: Distinguishing characteristics of normal and cancer cells

In general, normal cells have a regular and ellipsoid shape while

cancer cells are often irregular and contoured. Decreased adherence in cancer cells can lead to disorganized cell spreading and fewer cell-cell contacts, as well as an overall chaotic appearance to the cell population. In contrast, normal cells will grow as a uniform layer of cells with many tight connections between neighboring cells. Structural changes to nuclear lamina proteins, which provide mechanical support to cells, can affect chromatin organization and alter gene expression. Cancer cells also often feature changes to cell structures. For example, the size of the endoplasmic reticulum and mitochondria often decreases, the Golgi apparatus is under-developed, and the number of peroxisomes increase (Figure 4).

Histologists and pathologists routinely use these physical features to identify cancer cells within a patient's tissue sample. For diagnosis, the suspected cancerous tissue is biopsied and then fixed by a chemical or physical procedure to preserve the cells. The fixed tissue is then hardened, cut into very thin sections (one-cell thick), and placed onto a microscope slide. Finally, the prepared sections are treated using a variety of dyes that specifically stain the cellular structures. Methylene blue and eosin are two common dyes used to identify specific cellular features: methylene blue stains the nuclear material a deep blue color, while eosin will stain the cytoplasm and connective tissue a lighter pink (Figure 5). Together, these dyes allow a histologist to quickly and easily observe changes in cell structure and composition.

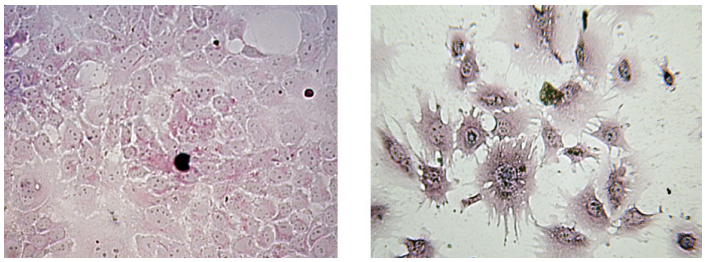


Figure 5: Example of Methylene blue and Eosin staining.

For this activity, students will rehydrate and stain pre-fixed cells provided on a glass slide. They will observe and analyze the morphological differences between normal cells and cancer cells using a compound microscope. Upon completion, the students will be able to describe the physical differences between normal and cancer cells and will understand the functional significance of these structural changes.

Experiment Overview

EXPERIMENT OBJECTIVE:

In this lab, students will explore the morphology of normal and cancerous cells by staining pre-fixed cells with methylene blue and eosin. The cell morphology will then be examined using light microscopy. After comparing the two cell types, students will identify the cancerous cells.

LABORATORY SAFETY

1. Wear gloves and goggles while working in the laboratory.
2. Always exercise extreme caution when working in the laboratory.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.
4. Always wash hands thoroughly with soap and water after working in the laboratory.
5. If you are unsure of something, ASK YOUR INSTRUCTOR!

Wear gloves and safety goggles

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

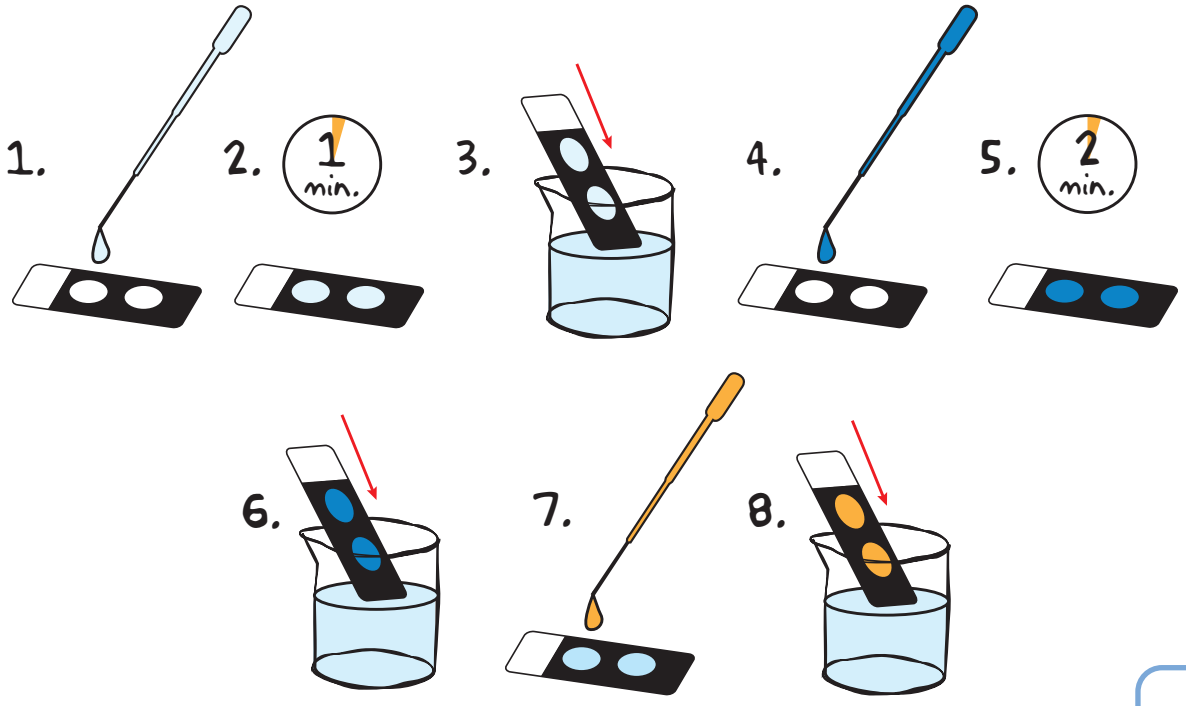
During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Module I: Staining the Pre-fixed Cells



Wear gloves and safety goggles

NOTE: Before beginning the experiment, ensure that the slide is facing upright.

1. Using a transfer pipet, **COVER** each well with rehydration buffer.
2. **INCUBATE** the slide for 1 minute at room temperature.
3. **RINSE** the slide briefly by submerging in the beaker of distilled water. Gently tap the slide on a paper towel to remove excess water.
4. Using a fresh transfer pipet, **COVER** each well with methylene blue stain.
5. **INCUBATE** the slide for 2 minutes at room temperature.
6. **RINSE** the slide briefly by submerging in the beaker of distilled water. Gently tap the slide on a paper towel to remove excess water.
7. Using a fresh transfer pipet, **COVER** each well with eosin stain.
8. Immediately **RINSE** the slide by submerging in the beaker of distilled water. If residual stain remains, change water and repeat until the water no longer turns orange. Gently tap the slide on a paper towel to remove excess water. **PROCEED** to Module II: Microscopic Observation.

OPTIONAL STOPPING POINT:
At this point, the stained slides can be stored at room temperature. If a coverslip is required by your microscope, one can be added by following the instructions in Appendix A.

Module II: Microscopic Observation

1. **LOCATE** cells in well #1 (Figure 6, below) using the lowest magnification objective. Adjust the slide to find a random field of nicely stained cells that contains at least 15-20 cells.
2. **DESCRIBE** the overall morphology of the cells in the table on the following page. Features you might record include the number of cells, overall cell morphology, shape and size of the nucleus, and the intensity or color of staining.
3. Using the space provided, **DRAW** an image of the field of cells that you observe.
4. **MOVE** the slide and observe a second field of cells. Are your observations from the first area consistent with the second field?
5. **SWITCH** to a higher magnification and record your observations as in steps 2-3.
6. **CHANGE** the microscope back to the lower magnification objective and repeat steps 1-5 for the second cell type in well #2.
7. Based on your observations, **CLASSIFY** each cell type as normal or cancerous.

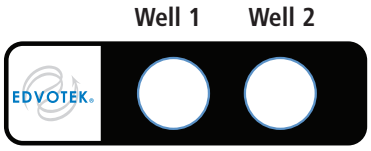


Figure 6: Diagram of ready-to-stain slide.

Module II: Microscopic Observation, continued

Well	Magnification	Observations	Drawing

Classification:

Well #1:_____ Well #2:_____

Study Questions

1. What is cancer?
2. What is the difference between an oncogene and a tumor suppressor?
3. Describe the characteristics of cancer cells that make them easy to culture in the laboratory.
4. How do pathologists distinguish between normal and cancer cells?
5. Why would you use two different dyes when staining cells?

Instructor's Guide

This lab is designed for six groups of 2-4 students.

This lab explores the differences in morphology between normal and cancerous cells. Students will work in groups to stain pre-fixed mouse mammary cells with methylene blue and eosin. The samples are examined using light microscopy, allowing students to identify the distinguishing characteristics of cancer cells through exploration. Common differences between normal and cancerous cells include cell growth, shape, and behavior.

It is important to remind students that natural variations can occur in cultured cells, both within a single well and when comparing the same condition across multiple slides. In addition, differences in staining technique or timing can alter the intensity of staining from group to group. When examining the slides, students should comment on general trends in each cell type. A pre-stained slide has been provided to serve as a reference for the expected staining and to help calibrate microscopes before beginning the experiment.

PRE-LAB PREPARATION

Pre-lab preparation should take **approximately 30 minutes** and can be performed any time before the lab period.

1. Label eighteen (18) 1.5 mL snap-top microcentrifuge tubes as follows:
   - 6 – Rehydration buffer
   - 6 – Methylene blue stain
   - 6 – Eosin Stain

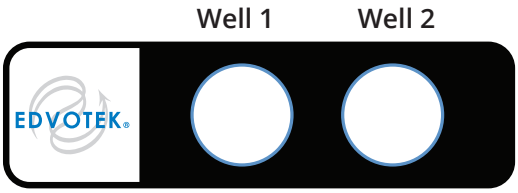
2. Use a separate transfer pipet for dispensing each component into the appropriately labeled tube.
   - Add approximately 0.5 mL of each solution to the tube.
   - Cap the tubes and store at room temperature.

3. Prepare beakers and distilled water for washing slides. The water level must be high enough to fully submerge the slides. If beakers are not available, slides can be gently washed under running water.

4. Distribute the following to each student group, or set up a workstation for students to share materials:
   - 1 Ready-to-Stain slide
   - Rehydration buffer
   - Methylene blue stain
   - Eosin stain
   - 3 Transfer pipets
   - 1 Pair of forceps (optional)
   - Beaker of distilled water
   - Paper towels or Kimwipes
   - Microscope

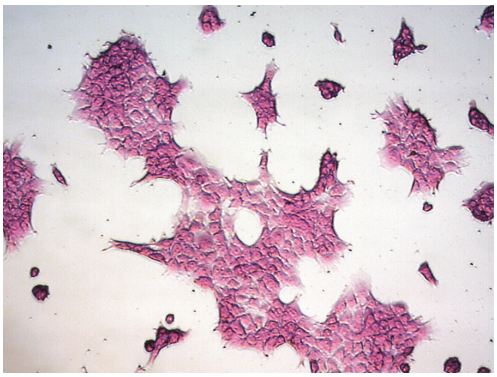
Experiment Results and Analysis

The cells in Well #1 (closer to the label) are normal mouse cells, while the cells in Well #2 are a mouse cancer cell line. Sample cell images are shown below for both types of cells. These images represent the typical results achieved from these cells; student results will vary due to slight fluctuations in cell preparation and the intensity of staining.

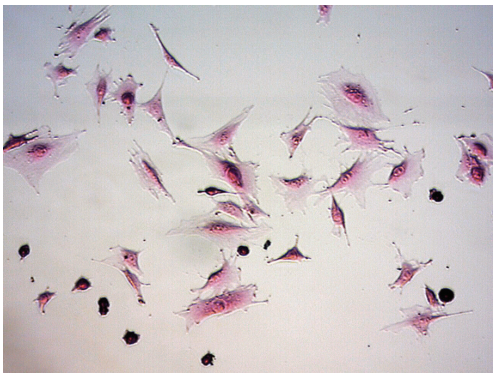


Students should be able to clearly observe the nuclei and cytoplasm of both cell types (see below for examples). In addition, differences in spreading, cell-cell contacts, and contact inhibition should be observed.

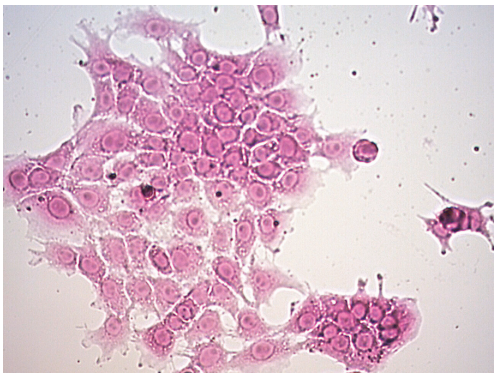
Normal Cells 40X



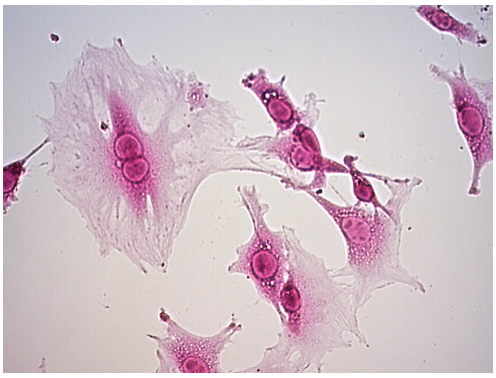
Cancer Cells 40X



Normal Cells 100X



Cancer Cells 100X



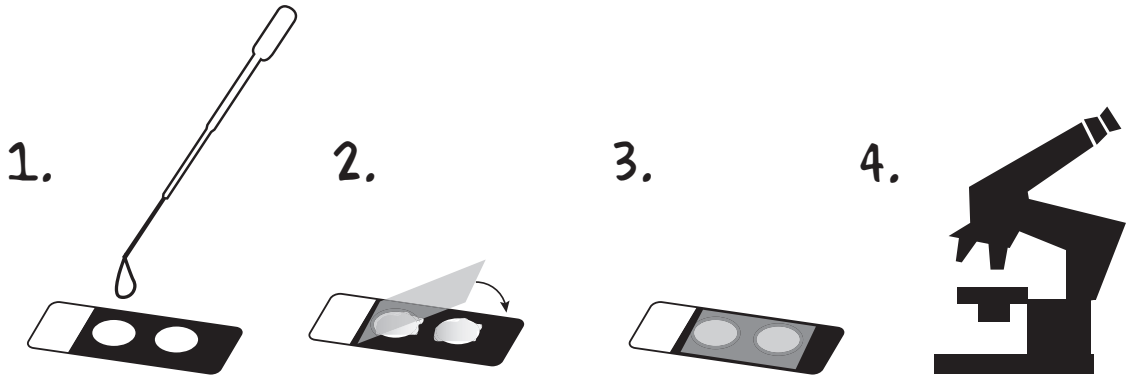
Cell type	Cell Shape	Spreading	Cell-Cell Contacts	Nuclei
Normal Epithelial Cells	Uniform, compact	Some spreading, but more compact. Monolayer.	Many cell-cell junctions	Uniform, small
Cancer Epithelial Cells	Random shapes, larger cells, disorganized arrangement.	Very spread cells, does not form uniform monolayer.	Fewer cell-cell junctions	Random, larger

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

Appendix A

Mounting Glass Coverslips (Optional)

NOTE: Mounting medium and slide coverslips are not included with this kit.



Glass coverslips may be required for some microscope objectives and can help to increase the visibility of nuclei and organelles on these microscopes. The mounting media will remove non-specific background stain, but can also cause lightly stained cells to fade. Because of this we recommend observing slides without a coverslip unless necessary. If time allows students can visualize slides before and after adding mounting media and coverslips.

Wear gloves and safety goggles

ADDING A COVERSLIP

1. Using a fresh transfer pipet, **ADD** 1 small drop of mounting medium to each well.
2. Carefully **PLACE** a coverslip on top of the mounting medium to cover both wells. **HINT:** Avoid bubbles by placing the cover slip and at 45° angle to the slides and slowly lowering. If bubbles are seen, gently press on the coverslip to displace.
3. Gently **ADJUST** the coverslip so that it is centered on the slide and fully covers both wells.
4. **PROCEED** to Module II: Microscopic Observation.

OPTIONAL STOPPING POINT:
Once the coverslip has been placed, the stained slides can be stored at 4°C for up to 24 hours before moving to Module II.