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Edvo-Kit # 987

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Chromosome Spread

Experiment Objective:

In this experiment, students will use Giemsa staining to examine the karyotype of cancer cells.

See page 3 for storage instructions.

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

Experiment Components

All components can be stored at room temperature.

Component	Check (✓)
• Ready to stain slides	❑
• Giemsa stain solution	❑

This experiment is designed for 6 groups.

Supplies

• Transfer pipets	❑
• Microcentrifuge tubes	❑

Requirements *(Not included with this kit)*

- Microscopes (400X total magnification recommended)
- Forceps
- Kimwipes
- Distilled water
- Gloves
- Beakers
- Slide coverslips (optional)
- Mounting medium (optional)

Background Information

UNDERSTANDING CHROMOSOMES

Chromosomes, strands of condensed DNA and protein, are found in the nucleus of almost every cell in our bodies. Each chromosome is composed of double-stranded DNA that is tightly wrapped around proteins known as histones, forming a complex known as chromatin (Figure 1). This arrangement of chromatin is essential for packaging the DNA molecules; unwrapped DNA is too long to easily fit into the nucleus and could be damaged. Instead, chromosomes allow eukaryotic cells to compactly store DNA, greatly reducing the overall length. In addition to providing structure, chromosomes help to regulate gene expression by hiding or uncovering segments of DNA, altering the rate of transcription for individual genes.

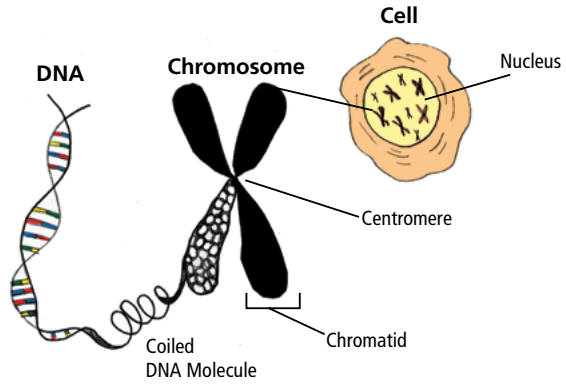


Figure 1: Chromosome Structure

Mitosis is one of the key stages of the cell cycle, encompassing the division of the nucleus, cytoplasm, and cell membrane to create two identical daughter cells. Although the process is tightly regulated and highly complex, mitosis represents only a small portion of the cell cycle. Instead, most cells spend the majority of their time in interphase, the G1, S, and G2 phases of the cell cycle, using this time to grow, duplicate DNA, and prepare for cell division (Figure 2). Next, the cell passes through an irreversible "checkpoint" and enters mitosis, rapidly moving through a number of phases:

- <u>Prophase:</u>
  The chromosomes are tightly condensed and mitotic spindles begin to form from the centrosomes. The nucleus starts to dissolve.

- <u>Metaphase:</u>
  Microtubules have attached to chromosomes and the fully condensed chromosomes align on the metaphase plane.

- <u>Anaphase:</u>
  Sister chromatids are separated and pulled to opposite ends of the cell.

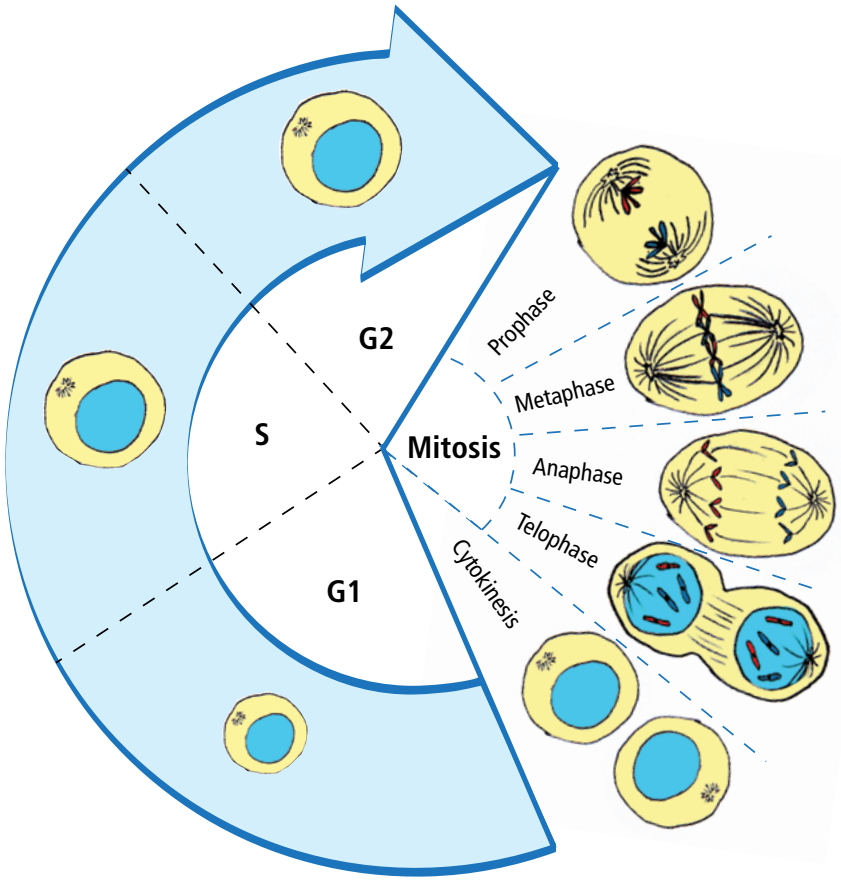


Figure 2: Schematic of the cell cycle.

- **Telophase:**
  The nuclear envelops reform, chromosomes begin to relax.

- **Cytokinesis:**
  The cell membrane "pinches" together between the two nuclei, producing two daughter cells.

The nuclei of normal human somatic cells each contain 23 pairs of chromosomes, each composed of two sister chromatids linked together at the centromere. During reproduction, one chromosome from each pair is derived from either maternal or paternal origin. In humans, the autosomes, or non-sex chromosomes, have historically been numbered from 1 to 22 in an approximation of decreasing size. The 23rd pair represents the sex chromosomes, referred to as X and Y; normal females contain two X chromosomes per nucleus, whereas normal males contain one X and one Y chromosome. It is vital that each of these chromosomes is properly segregated during mitosis and meiosis -- DNA encodes the instructions necessary for cell behavior and survival, and any abnormalities in the number or composition of chromosomes can lead to disease.

Chromosomal Abnormalities Can Lead to Disease

Variations in the normal complement of chromosomes have been associated with numerous prenatal diseases. This can include numerical alterations, where chromosomes are improperly gained or lost, or structural alterations such as deletions, duplications and translocations. Approximately 0.5% of all live births are associated with some form of chromosomal abnormality (Table 1).

TABLE 1: Common Chromosomal Abnormalities

Chromosome	Abnormality	Disease
5	5p deletion	Cri-du-Chat
7	7q deletion	William's syndrome
8	Trisomy	Warkany syndrome
8	8q deletion	Langer-Giedon syndrome
9	Trisomy	Rethore syndrome, Trisomy 9 syndrome
9	9p deletion	Alfi's syndrome
11	Deletion	11p- Wilms tumor, 11q- Jacobson syndrome
13	Trisomy	Patau's syndrome
15	15q deletion	Prader-Willi, Angelman's syndrome
16	Trisomy	Fatal in early development
17	Trisomy (17p duplication)	Charcot-Marie-Tooth disease
18	Trisomy	Edward's syndrome
21	Trisomy	Down's syndrome
22	Trisomy	Trisomy 22 syndrome
22	22q deletion	DiGeorge syndrome
X	Monosomy	Turner's syndrome
X	Duplication	XXY-Klinefelter, XXX-Trisomy X, XXXX-Four X
Y	Duplication	Double Y syndrome

The most common of these chromosomal genetic disorders is Down's syndrome, found in 0.125% of live births, which is due to the partial or complete duplication of chromosome 21 (Figure 3). This results in trisomy 21, the presence of three copies of the 21st chromosome. Additional trisomies, such as chromosome 13 (Patau's syndrome) and 18 (Edward's syndrome) are less common in live births, and trisomies for every other autosome have been documented in miscarried fetuses.

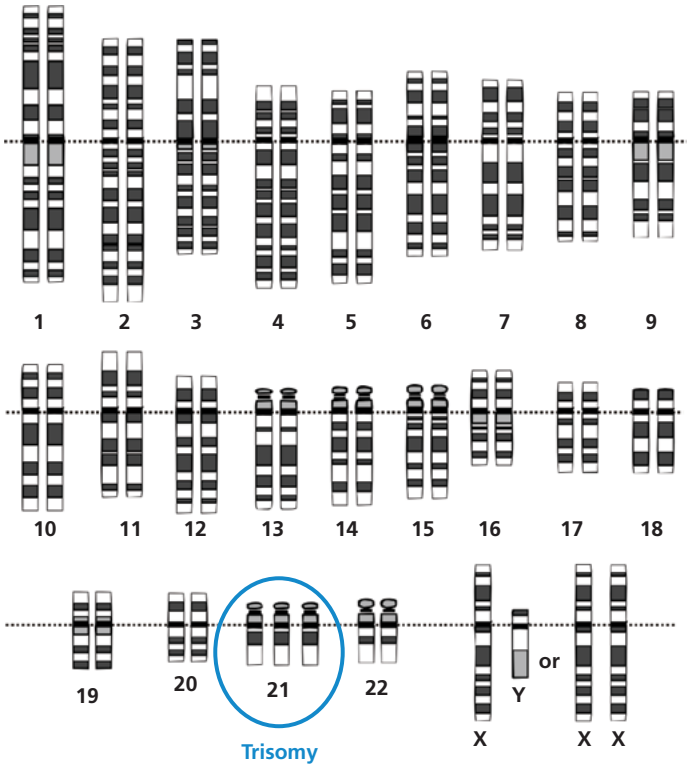


Figure 3: Down's syndrome karyotype

Deletion of whole or partial chromosomes has also been linked to disorders. A partial deletion in the short or "p" arm of chromosome 5 is responsible for Cri-du-Chat syndrome ("Cry of the cat"), a disease named for the characteristic cat-like cry of affected children (Figure 4). In contrast, deletions of entire autosomes are usually not tolerated and result in miscarriage, although single copies (monosomy) for chromosomes 21 and 22 have occasionally been reported. The reason for poor tolerance of these monosomies is not entirely known, although it seems reasonable that large decreases in gene expression would lead to problems in development.

In contrast to autosomes, deletions or duplications of sex chromosomes are better tolerated in developing fetuses. This is mainly due to the fact that all but one of the X chromosomes in each cell is inactivated early in development, as well as the limited number of genes on the Y chromosome. Thus, monosomy X (Turner syndrome), as well as XXX, XXY, XYY and even XXXXXX karyotypes have been reported in live individuals, and can frequently be well tolerated with minimal symptoms. Other "abnormal" karyotypes can similarly give rise to normal healthy individuals. For example, balanced translocations, in which reciprocal regions of different chromosomes have been switched, are usually not dangerous (Figure 5). However, the offspring of these individuals run the risk of inheriting one of the rearranged chromosomes.

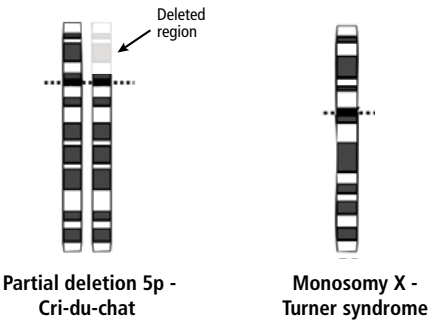


Figure 4: Partial and complete chromosomal deletions

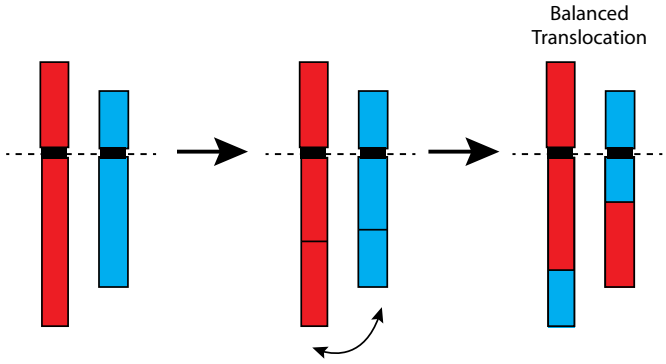


Figure 5: Balanced translocation between chromosomes

Chromosomal Abnormalities in Cancer

Chromosomal analysis is also used to analyze certain types of cancers. Leukemia, lymphomas, and many sarcomas are characterized by specific chromosomal translocations. These alterations lead to the activation of oncogenes or the formation of oncogene fusion proteins. The first specific abnormality described for a human cancer was the "Philadelphia" chromosome found in most chronic myelogenous leukemia (CML) patients. This chromosomal abnormality is due to a reciprocal translocation between chromosomes 9 and 22, resulting in the fusion of the c-ABL proto-oncogene and the BCR gene (Figure 6). Many other cancers feature chromosomal alterations, including deletions of regions encoding tumor suppressors and DNA repair genes, or rearrangements that lead to increased oncogene function.

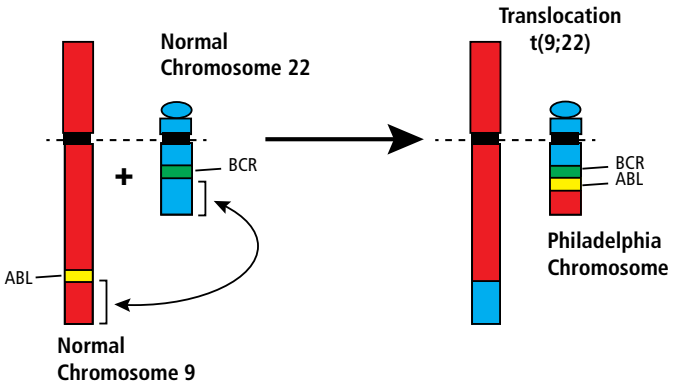


Figure 6: The Philadelphia chromosome

In addition, studies show that the majority of human cancers have gained or lost whole chromosomes as the result of increased genetic instability. The average colon, breast, pancreas, or prostate cancer cell can lose up to one-fourth of its genetic material. At the same time, the chromosomal rearrangements in cancer can lead to abnormal mitosis, resulting in aneuploidy, an abnormal number of chromosomes. It is not uncommon for tumor cells to possess two to three times as many total chromosomes as healthy cells.

The cells used in this experiment are an immortalized CML cell line that has been grown in laboratories for decades, further contributing to genomic instability. Thus, these cells display a highly abnormal karyotype; most of the cells feature the Philadelphia chromosome, a second translocation between chromosomes 15 and 17, and up to 68 total chromosomes.

Detecting Chromosomal Abnormalities - The Karyotype

Karyotypes have become one of the primary tools used in the detection of chromosomal diseases. To perform a karyotype, cells are cultured briefly in the laboratory and then treated with the drug colchicine, a microtubule inhibitor. During the metaphase stage of mitosis, condensed chromosomes are normally aligned at the metaphase plate and prepared for separation by spindle microtubules. However, colchicine treated cells cannot form microtubules, forcing the cells to arrest before division. The arrested cells are then fixed and adhered to a microscope slide. Finally, the slides are incubated with Giemsa stain, a mixture of dyes that selectively stains DNA blue, allowing the chromosomes to be easily visualized by microscopy.

This experiment familiarizes students with the basic principles of microscopy and the study of chromosomes. Pre-fixed slides containing metaphase arrested cancer cells are incubated with Giemsa stain to reveal condensed chromosomes, allowing for the analysis of chromosome number and morphology

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will use Giemsa staining to examine the karyotype of cancer cells.

LABORATORY SAFETY

Wear gloves and safety goggles

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!

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.

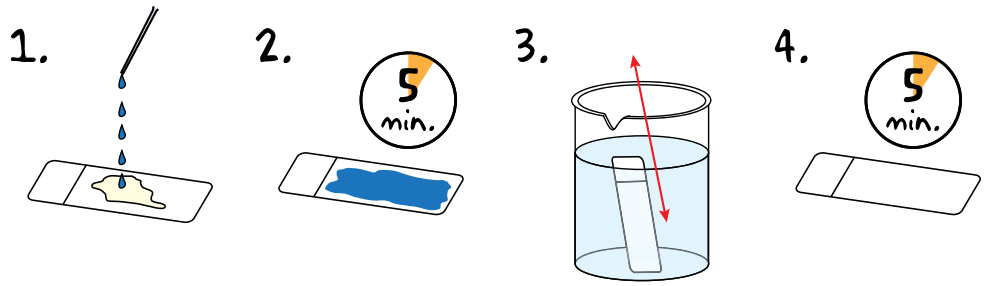
During the Experiment:

- 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 Procedure



1. Using a transfer pipet, **ADD** the entire volume of Giemsa stain to the area of the slide containing the metaphase spreads. Try to cover the slide without letting the stain overflow.
2. **INCUBATE** the slide for 5 minutes at room temperature.
3. **RINSE** the slide by briefly submerging in a beaker of distilled water. Gently **TAP** the slide on a paper towel to remove excess water. If residual stain remains, change the water in the beaker and repeat rinsing the slide until the water no longer turns blue.
4. **AIR DRY** the slide for 5 minutes.

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.

HINTS:
Slides can be washed in a beaker of distilled water by gently submerging for 30 seconds. If residual stain remains, change water and repeat wash step until water no longer turns blue.

Avoid bubbles by placing the cover slip at 45° angle to slide and gently lowering. If bubbles are seen, gently press on the cover slip to displace.

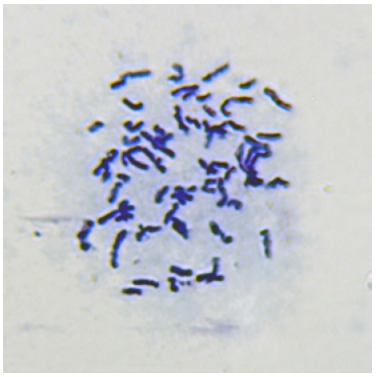
Module II: Microscopic Observation

1. Using the 10/20X objective, locate cells with good metaphase spreads. Look for a field that is nicely stained and contains well spread, non-overlapping chromosomes.

2. Move to the 40/100x objective and count the number of chromosomes in the field of view. Make note of distinguishing chromosomal features including the presence of centromeres or abnormal chromosomal structures, if the cell contained individual chromosomes or paired chromatid (X-shaped, replicated chromosomes), and other noteworthy observations. Record the data below or in your lab notebook.

3. Focus on a different field of cells and repeat the observation an additional four times.

4. Tabulate your data for the observed chromosomes in the grid provided.



Field	Total Chromosome#	Observations
1		
2		
3		
4		
5		

Study Questions

1. What is the normal chromosomal number for human cells? Are these cells normally aneuploid or diploid?
2. How does colchicine work in cells and why is it useful for metaphase spreads?
3. Why do only some of the cells display chromosomes while others contain intact nuclei?
4. Why are certain chromosomal abnormalities severely detrimental or lethal, while other abnormalities are relatively mild?
5. Why do balanced translocations between two chromosomes still often result in healthy individuals? How can such a translocation lead to an abnormal phenotype in the offspring even if the parents are both healthy?

Instructor's Guide

PRE-LAB PREPARATION

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

1. Label six 1.5 mL snap-top microcentrifuge tubes as "Giemsa stain". Dispense 400 µL of Giemsa stain into the tubes. Cap the tubes and store at room temperature.

2. Prepare beakers with distilled water for washing slides. If beakers are not available, slides can be gently washed under running water.

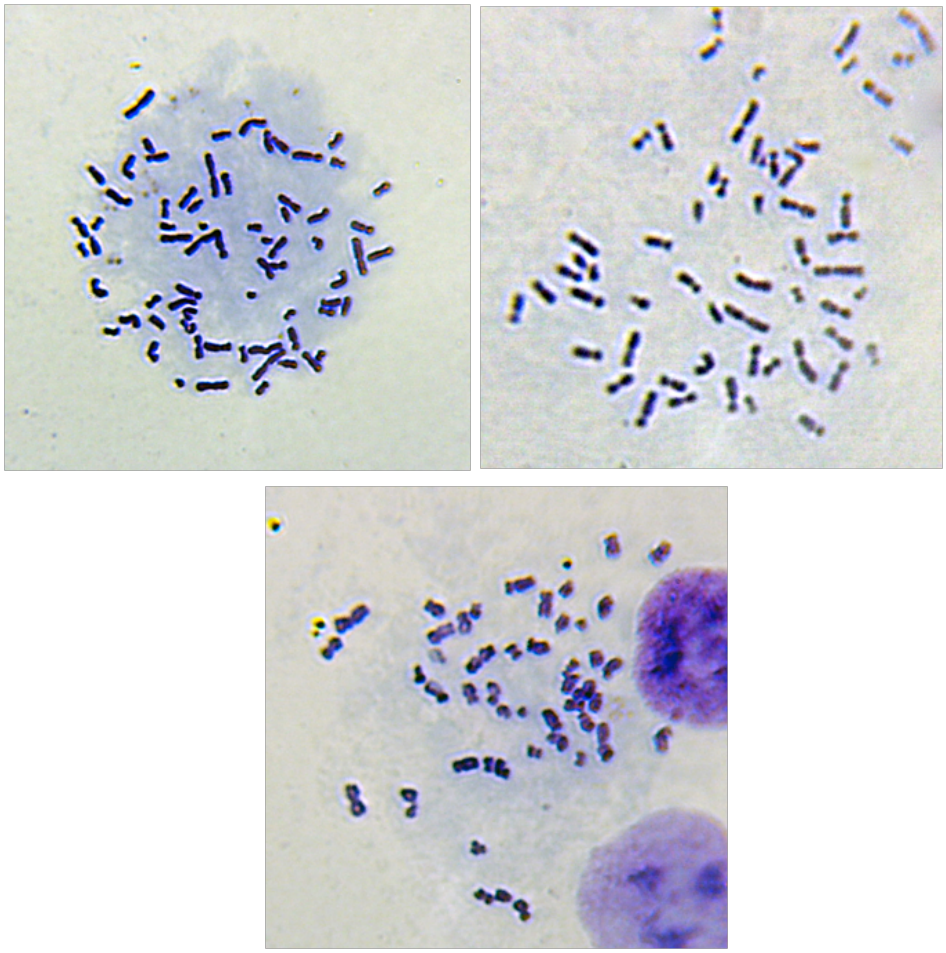
3. Distribute the following to each student group, or set up a workstation for students to share materials.
   - 1 Ready-to-stain slide
   - Giemsa stain
   - 2 Transfer pipets
   - 1 Pair forceps
   - Beaker of distilled water
   - Kimwipes

NOTE: Rarely, microscopes will require a coverslip/cover glass for best results. Coverslips and mounting medium are not included in this experiment - please refer to the manual included with your microscope for specific information on the required coverslips.

Experiment Results and Analysis

Sample metaphase chromosome spreads are shown below for the immortalized CML cells. These images represent the typical results achieved from these cells, which contain numerous translocations and disorganized chromosomes. Chromosome counts will indicate up to 68 chromosomes.

Banding patterns will not be observed with standard Giemsa staining, but features such as chromosome length and centromere location can often be detected.

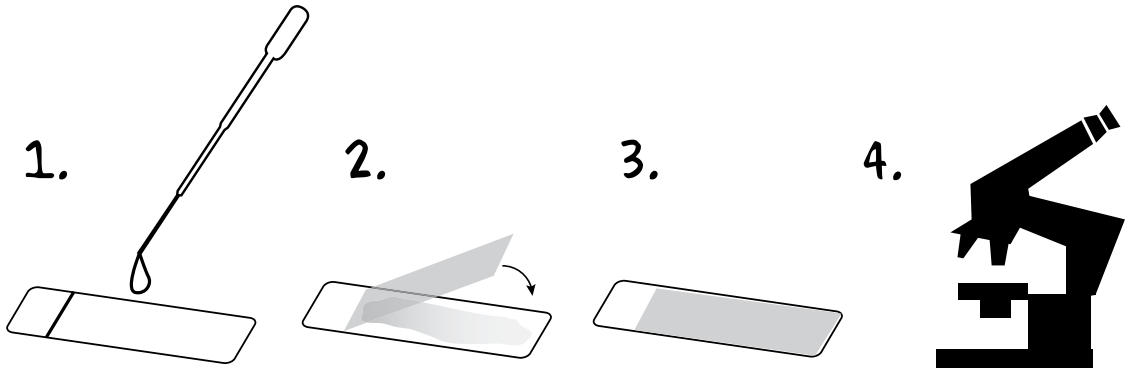


Please refer to the kit insert for the Answers to Study Questions

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. Occasionally, mounting media can 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** 2 small drops of mounting medium to the middle of the slide.
2. Carefully **PLACE** a coverslip on top of the mounting medium to cover the slide. **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.
4. **PROCEED** to Module III: Microscopic Observation.

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