

Cell Biology Cell Staining: Cheek Cells

Grades 9-12

Objective

By performing this lab investigation, students will:

1. Observe and identify cells and cell structures
2. Become familiar with and perform simple cell staining techniques
3. Perform wet mounting of samples onto a glass slide
4. Become familiar with and be able to use a microscope

Lab Introduction

The building block of all living organisms is the cell. Some organisms, such as bacteria, are unicellular while other organisms, such as plants and animals, are multicellular. Each living cell can take in nutrients, convert these nutrients into energy, carry out specialized functions, and reproduce as necessary. Each cell stores its own set of instructions (genes) for carrying out each of these activities. Suggested further detailed discussions on cellular activities include genes, transcription/translation, replication and cell division (mitosis/meiosis), metabolism or metabolic pathways, and responses to stimuli (internal and external).

The two basic types of cells are called prokaryotic and eukaryotic. All cells, whether prokaryotic or eukaryotic, have a membrane that envelops the cell, separates its interior from its environment, regulates what moves in and out (selectively permeable), and maintains the electrical potential of the cell. Inside the membrane, a cytoplasm takes up most of the cell volume. All cells possess DNA, the hereditary material of genes, and RNA, containing the information necessary to build various proteins such as enzymes, the cell's primary machinery. There are also other kinds of biomolecules in cells.

To better visualize cells and cell structures under a microscope, cell staining techniques are used. Certain cell components, such as the nucleus, cell wall, or the entire cell can be preferentially stained using different reagents. Since cells also regulate intake of nutrients or biomolecules, exclusion or retention of certain stains can also serve as indicators of cell viability. One such assay is the Trypan Blue Exclusion Assay that is routinely used in research laboratories to quantify total numbers of cells as well as to quantify live versus dead cells. Live or living cells will actively pump out, or exclude, Trypan Blue stain while dead cells cannot exclude the dye and will show as distinctively blue under a microscopic examination.

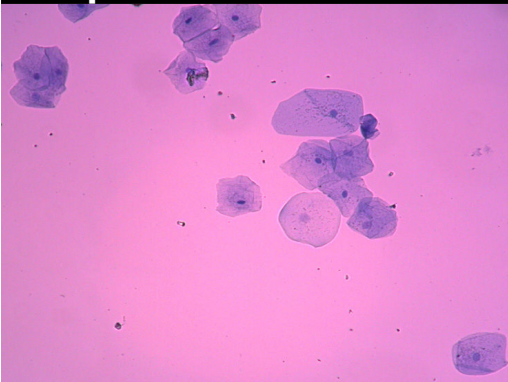
Materials

1. Gloves
 2. Safety glasses
 3. Lab coats (if available)
 4. Clean toothpicks used to scrape cheek cells
 5. Clean glass slides (VWR catalog #82027-130)
 6. Clean cover slips (VWR catalog #16004-308)
 7. Clean cellTRAY® Dishes (Nanopoint CA-T-30)
 8. Trypan Blue (VWR catalog #VW17-942E)
 9. Microscope with 4x to 10x objectives
- Optional:* 15mL drop-dispensing bottles (VWR catalog #2411-0015)

Procedure

1. Aliquot Trypan Blue solution into individual drop-dispensing bottles to avoid waste and cross-contamination of stock solutions.
2. Distribute gloves, toothpicks, glass slide, cover slip, and Trypan blue.
3. Students should don gloves, safety glasses, and lab coats (if available) to prevent staining hands and clothing.
4. Place slide in cellTRAY dish.
5. Place a single drop of Trypan Blue on the glass slide. **Caution: Trypan Blue stain can be cytotoxic to cells, avoid ingestion and eye splashes.**
6. Scrape, with the flat, wide end of the toothpick, the insides of the cheeks.
7. Smear or stir the scrapings in the drop of stain, spread the stain to a 1" x 1" square (approx).
8. Position a cover slip over the top of the stain, lower an edge of the cover slip onto the edge of the drop of stain, then gently lower the rest of the cover slip down onto the drop of stain.
9. Observe using a microscope starting with the scanning objective and working up to the high power objective.

Expected Observation



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NSES Standards

Science as Inquiry: Content Standard A

1. Identify questions and concepts that guide scientific investigations
2. Design and conduct scientific investigations

Life Sciences: Content Standard C

1. Cells are enclosed within semi permeable membranes that regulate their interaction with their surrounding

Science and Technology: Content Standard E

1. Science often advances with the introduction of new technologies

Student Observation

1. Make drawings of the cells and label all the structures you see.
2. Clean and dry the slide after your observations and data collection.
3. Make sure that your lab station is clean and that the microscope is not left on high power, that it is unplugged and covered, that the slides have been cleaned and are dry and the station is ready for the next team.

Discussion

1. What is the ratio or percentage of live versus dead cells?
2. Why are most of the cheek cells stained blue?
3. What is the expected ratio of live versus dead cells in a cancerous tumor?

Cell Biology Cell Staining: Skin Cells

Grades 9-12

Objective

By performing this lab investigation, students will:

1. Observe and identify cells and cell structures
2. Become familiar with and perform simple cell staining techniques
3. Perform wet mounting of samples onto a glass slide
4. Become familiar with and be able to use a microscope

Lab Introduction

The building block of all living organisms is the cell. Some organisms, such as bacteria, are unicellular while other organisms, such as plants and animals, are multicellular. Each living cell can take in nutrients, convert these nutrients into energy, carry out specialized functions, and reproduce as necessary. Each cell stores its own set of instructions (genes) for carrying out each of these activities. Suggested further detailed discussions on cellular activities include genes, transcription/translation, replication and cell division (mitosis/meiosis), metabolism or metabolic pathways, and responses to stimuli (internal and external).

The two basic types of cells are called prokaryotic and eukaryotic. All cells, whether prokaryotic or eukaryotic, have a membrane that envelops the cell, separates its interior from its environment, regulates what moves in and out (selectively permeable), and maintains the electrical potential of the cell. Inside the membrane, a cytoplasm takes up most of the cell volume. All cells possess DNA, the hereditary material of genes, and RNA, containing the information necessary to build various proteins such as enzymes, the cell's primary machinery. There are also other kinds of biomolecules in cells.

To better visualize cells and cell structures under a microscope, cell staining techniques are used. Certain cell components, such as the nucleus, cell wall, or the entire cell can be preferentially stained using different reagents. Since cells also regulate intake of nutrients or biomolecules, exclusion or retention of certain stains can also serve as indicators of cell viability. One such assay is the Trypan Blue Exclusion Assay that is routinely used in research laboratories to quantify total numbers of cells as well as to quantify live versus dead cells. Live or living cells will actively pump out, or exclude, Trypan Blue stain while dead cells cannot exclude the dye and will show as distinctively blue under a microscopic examination.

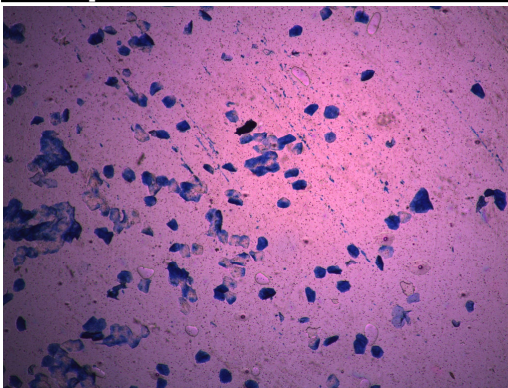
Materials

1. Gloves
 2. Safety glasses
 3. Lab coats (if available)
 4. Clean clear cellophane tape
 5. Clean glass slides (VWR catalog #82027-130)
 6. Clean cellTRAY® Dishes (Nanopoint CA-T-30)
 7. Trypan Blue (VWR catalog #VW17-942E)
 8. Microscope with 4x to 10x objectives
- Optional:* 15mL drop-dispensing bottles (VWR catalog #2411-0015)

Procedure

1. Aliquot Trypan Blue solution into individual drop-dispensing bottles to avoid waste and cross-contamination of stock solutions.
2. Distribute gloves, glass slide, cellTRAY Dish, and Trypan Blue.
3. Students should don one glove, safety glasses, and lab coat (if available) to prevent staining hands and clothing.
4. Place slide in cellTRAY Dish.
5. Place a single drop of Trypan Blue on the glass slide. **Caution: Trypan Blue stain can be cytotoxic to cells, avoid ingestion and eye splashes.**
6. Place a small strip of clear tape to the palm of the students' hand. Rub the tape on the skin for a few seconds so that skin cells adhere to the tape.
7. Peel the tape from one corner; try not to touch the side that was in contact with the skin.
8. Place the tape over the drop of Trypan Blue.
9. Observe using a microscope starting with the scanning objective and working up to the high power objective. **Note:** No live cells will be observed; skin cells will appear smaller than cheek cells; nuclei will be harder to distinguish.

Expected Observation



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Discussion

1. What is the ratio or percentage of live versus dead cells?
2. Why are most of the hand cells stained blue?
3. What is the expected ratio of live versus dead cells in a cancerous tumor?