Basic Stain Set

Introduction

Biological stains and staining techniques are indispensable in the fields of microscopy, cytology, histology, pathology and microbiology—to name a few! They provide the beginning biologist tools for making the invisible visible. Familiarity with several stains and some basic techniques are skills that will be relied upon time and time again. This Basic Stain Set includes several of the most commonly used stains from beginning to advanced biology. Each is listed with most common uses and applications. Uses are not limited to those described.

Background

Lugol's iodine one of the most useful general-purpose stains in the biology lab. It is used for plant and animal tissue cells—such as onion skin and cheek cells. Lugol's iodine reveals flagella, cilia and nuclei and also stains glycogen a reddish brown color. It is also widely used to test for starch molecules in leaves and food samples.

Another of the most popular general-purpose stains is methylene blue. Used for bacteria, yeasts and fungi, and for plant and animal tissue cells. As a bacterial stain it can be used to show endospore formation—staining cell contents blue while spore remains unstained. Bacterial smears should be stained for one to two minutes. As a general cellular stain it will show nuclei, chromatin granules and cytoplasmic granules. To use as a vital stain for live protozoa it should be diluted as low as 1:1,000—try one to ten milliliters of staining solution diluted up to one liter.

Methyl green is most useful for protozoans and unicellular algae. Also useful as a vital stain for live protozoans. In protozoa it will stain chromatin bright green, extranuclear structures blue or violet, and nucleoli will remain unstained. For use as a vital stain it must first be diluted to a harmless concentration. To do so, dilute 5–10 mL of 1% solution up to a liter with distilled or deionized water.

Eosin Y, 1% alcoholic is used most frequently for staining dead protozoans. Functions as a plasma/cytoplasmic stain. Also used as a background stain to enhance contrast between organism and background. Applied as a counter stain to basic dyes, such as methylene blue.

The most widely used stain for blood smears is Wright's staining solution. To use, begin by preparing thin smears on scrupulously clean slides—smears should be air dried. Apply 1 mL of stain to smear and let stand for 1–3 minutes. Apply 2 mL distilled water or phosphate buffer for 2–5 minutes. Finally rinse with distilled water or phosphate buffer. The phosphate buffer solution should be in a pH range of 6.4 to 6.8 and acts as a decolorizer/rinse agent. A pH in this range is recommended for optimal coloration of all blood cell types.

Sudan III is a fat-soluble stain—it is not soluble in water. Will stain neutral fats a distinctive red.

Materials

- Eosin Y, 1% Alcoholic
- Lugol's Iodine
- Methyl Green, 1% Aqueous Acidified
- Methylene Blue, Loeffler
- Sudan III, Alcoholic
- Wright's Staining Solution, 0.3% Alcoholic

Safety Precautions

Basic stains will stain skin as well as most materials. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. Dilute stains can be disposed of according to Flinn Suggested Disposal Method #26b.

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General Staining Tips

All of the included stains can be applied using a variety of techniques: (1) place a small drop of stain on the slide and allow to dry before adding drop of culture, (2) place drop of culture on slide—add drop of stain and coverslip, (3) add stain to edge of coverglass of prepared wet mount, (4) add stain directly to culture dish, (5) for dry mounts or flame-fixed smears, flood slide with stain or immerse in staining dish for one to several minutes. These techniques presume prior dilution of stock staining solution to recommended working strength. The technique selected will depend on the type of mount prepared.

References

Behringer, M. P. *Techniques and Materials in Biology*, 2nd ed.; Krieger: Malabar, Florida, 1989.

Morholt, E.; Brandwein, P. F. *A Sourcebook for the Biological Sciences*, 3rd ed.; Harcourt, Brace, Jovanovich: Orlando, Florida, 1986.

Staining Procedures, 4th ed.; Clark, G., Ed; Williams and Wilkins: Baltimore, 1981.

The Basic Stain Set is available from Flinn Scientific, Inc.

Catalog No.	Description
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Consult the Flinn Scientific website for current prices.