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AFB Ziehl Neelson Kit

Cat. #24669

Polysciences, Inc. provides many microbiological stain kits useful for a variety of chemical analyses. Each kit contains the necessary stains and dyes specific for the test. Please refer to our Material Safety Data Sheets (MSDS) for recommendations on personal protective equipment, material handling and waste disposal.

Kit Components

One 8 oz. bottle of the following:

1. Carbol Fuchsin (Ziehl Neelson)
2. Acid Alcohol
3. Methylene Blue

Introduction

Although no definitive evidence has been presented, the high lipid content (especially the mycolic acid component) of mycobacteria is thought to be related to the mechanism of acid fastness. Mycolic acids are long chain fatty acids found in the cell wall. Even light mechanical injury to the cell wall will cause bacteria to lose the “acid fast” characteristic, suggesting that permeation through cell membranes might be an important part of the mechanism. Carbol fuchsin is used to stain the slide and acid alcohol is used to decolorize the slide. It has been suggested that the dye replacement power of the counter stain could be used to decolorize and counter stain at the same time. Acid alcohol increases the differentiation obtained and gives superior results. After decolorization the slide may be counter stained with methylene blue or brilliant green. The “acid fast” organisms will appear red while non-“acid fast” organisms will appear blue or green.

Specimen Collection

Organisms being stained by an acid fast method are usually taken from a solid or a liquid medium on (in) which they have been cultured from their original source. An aqueous suspension is made (in the case of a solid medium) by taking a small amount of the material and suspending it in a drop of distilled water on a microscope slide. Care should be taken not to make the smear too thick. In the case of a liquid medium, a drop is used directly from the culture container. However, due to the solids from the medium, this method is not always satisfactory. The suspension made by either method is air dried and then “fixed” by passing it rapidly through a Bunsen burner flame two or three times. Allow the smear to cool before staining.

Procedure

1. Place the fixed smear on a staining rack and flood the slide with Ziehl Neelson stain. Heat the underside of the slide for 3 minutes. Do not allow stain to boil.
2. Wash off the stain with distilled water.
3. Decolorize with acid alcohol until no color runs from the smear.
4. Rinse thoroughly with distilled water.
5. Flood slide with methylene blue or brilliant green for 1-2 minutes.
6. Rinse thoroughly with distilled water and air dry.
7. Examine dry under high magnification and verify under oil immersion.

Sources of Error

1. Overheating (burning) during fixation can be avoided by just touching the back of the slide carefully to the back of the hand each time the slide has been passed though the flame.
2. Do not stain smears which have only been air dried. Smears must also be “fixed.”
3. Smears should not be too thick. After air drying, examine them under a microscope. If there are no areas of bacteria separation, more water should be added to dilute the smear.
4. After staining it is essential that the back surface of the slide is wiped clean.
5. If washing with distilled water is not done adequately, crystallization of the stain may appear on the slide.

Ordering Information

Cat #	Description	Size
24669	AFB Ziehl Neelson Kit	1 Kit

TO ORDER

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