

TECHNICAL DATA SHEET 749

Gram's Stain with Iodine

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Cat. #24668

Polysciences 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. Crystal Violet
2. Gram's Iodine
3. Decolorizer
4. Safranin

Introduction

The gram stain permits the separation of all bacterial species into two large groups, those which retain the primary dye (crystal violet), and those which lose the primary dye and take the color of the secondary dye (safranin). The mechanism of gram stain is based on the distinctive chemistry and physical properties of the cell wall, possibly the lipid content. However, the exact mechanism of gram staining has not been confirmed.

Gram staining is based on the ability of the bacteria cell wall to retain the crystal violet dye during solvent treatment. The cell walls for gram-positive microorganisms have a higher peptidoglycan and lower lipid content than gram-negative bacteria. Bacteria cell walls are stained by the crystal violet. Iodine is subsequently added as a mordant to form the crystal violet-iodine complex so that the dye cannot be removed. This is referred to as fixing the dye. However, subsequent treatment with a decolorizer, which is a mixed solvent of ethanol and acetone, dissolves the lipid layer from the gram-negative cells. The removal of the lipid layer enhances the leaching of the primary stain from the cells into the surrounding environment. In contrast the solvent dehydrates the thicker gram-positive cell walls, closing the pores as the cell wall shrinks during dehydration. As a result the diffusion of the violet-iodine complex is blocked, and the bacteria remain stained. The length of the decolorization is critical in differentiating the gram-positive bacteria from the gram-negative bacteria. A prolonged exposure to the decolorizing agent will remove all the stain

from both types of bacteria. Some gram-positive bacteria may lose the stain easily and therefore appear as a mixture of gram-positive and gram-negative (gram-variable). Finally a counterstain of safranin is used.

Specimen Collection

Organisms being stained by the gram method are usually taken from a solid or liquid medium on (in) which they have been cultured from their original source (e.g. wounds, throat, swabs, sputum, etc.). An aqueous suspension is made (in the case of the 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, then "fixed" by passing 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 cover completely with crystal violet for 30-60 seconds.
2. Wash off the stain with distilled water.
3. Cover the slide with iodine for 30 seconds.
4. Wash off with distilled water.
5. Decolorize with Gram decolorizer for 10-15 seconds.
6. Wash thoroughly with distilled water.
7. Cover completely with safranin for 30-60 seconds.
8. Wash with distilled water and air dry. Examine under immersion oil.

Sources of Error

1. Overheating (burning) during fixation can be avoided by carefully touching the back of the slide to the back of the hand each time the slide has been passed through 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 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
24668	Gram's Stain Kit with Iodine (6 mo. stability)	1/Kit
24667	Gram's Stain Kit with Stabilized Iodine (2yr. stability)	1/Kit
24682	Gram's Decolorizer (75/25)	1 Gallon
24683	Gram's Decolorizer (50/50)	1 Gallon
24684	Gram's Iodine	1 Gallon
24685	Gram's Iodine-Stabilized	1 Gallon

To Order:

In The U.S. Call: 1-800-523-2575 or 215-343-6484

In The U.S. Fax: 1-800-343-3291 or 215-343-0214

In Germany Call: (49) 6221-765767

In Germany Fax: (49) 6221-764620

Order online anytime at www.polysciences.com