

## 77730 Gram Staining Kit (Bacteria Staining Kit according to Gram)

The Gram staining method is one of the most important staining techniques in microbiology. It is almost always the first test performed for the identification of bacteria. The primary stain of the Gram's method is crystal violet. The microorganisms that retain the crystal violet-iodine complex appear purple brown under microscopic examination. These microorganisms that are stained by the Gram's method are commonly classified as gram positive bacteria. Others that are not stained by crystal violet are referred to as gram negative bacteria. Besides Gram's stain, there is a wide range of staining methods available. The procedures for these other methods follow quite closely those of Gram's stain. By using appropriate dyes, different parts of the cell structure such as capsules, flagella, granules, or spores can be stained. Staining techniques are widely used to visualize components under the light microscope, for the differentiation and identification of microorganisms. In this aspect, Gram's stain is one of the most important diagnostic tools in biological science.

### Reagents:

### Produkt No.

Gram's crystal violet Solution	94448
Gram's iodine Solution	90107
Gram's Decolorizer Solution	75482
Gram's safranin Solution	94635

### Directions:

#### *Prepare a Slide Smear:*

- Transfer a drop of the suspended culture to be examined on a slide with an inoculation loop. If the culture is to be taken from a Petri dish or a slant culture tube, first add a drop or a few loopful of water on the slide and aseptically transfer a bit of the colony. It should only be a very small amount of culture. A visual detection of the culture on an inoculation loop already indicates that too much is taken.
- Spread the culture with an inoculation loop to an even thin film over a circle of 1.5 cm in diameter. It is possible to put 3 to 4 small smears on a slide, if more than one culture is to be examined.
- Hold the slide with a clothes-pin. Allow to air dry and fix it over a gentle flame, while moving the slide in a circular fashion to avoid localized overheating. The applied heat helps the cell adhesion on the glass slide to make possible the subsequent rinsing of the smear with water without a significant loss of the culture.

#### *Gram Staining:*

1. Flood the fixed smear with Gram's crystal violet Solution. Let stand for 60 seconds.
2. Pour off the stain and gently wash with tap water from a faucet or a plastic water bottle.
3. Flood with Gram's iodine Solution. Allow it to remain for 60 seconds.
4. Pour off the iodine solution and gently wash with tap water. Shake off the excess water from the surface.
5. Decolorize with Gram's Decolorizer Solution until the blue dye no longer flows from the smear. Further delay will cause excess decolorization in the gram-positive cells, and the purpose of staining will be defeated.
6. Gently wash the smear with tap water.
7. Counterstain with Gram's safranin Solution for 60 seconds.
8. Wash off the red safranin solution with water. Blot with bibulous paper to remove the excess water. Alternatively, the slide may be shaken to remove most of the water and air-dried.
9. Examine the finished slide under a microscope (oil immersion objective).

Attention: Wash off any spilled stain immediately with water to avoid leaving permanent marks in the sink, lab bench, or glassware.



#### Result:

- Gram-positive organisms are bluish purple
- Gram-negative organisms are pinkish red

#### Principle and Interpretation:

Gram staining is based on the ability of bacteria cell wall to retaining 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 easily. This step is commonly 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 solvent. 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 bacteria (Gram-variable).

Finally, a counterstain of safranin is applied to the smear to give decolorized gram-negative bacteria a pink color. Some laboratories use a basic fuchsin solution (Cat. No. 87794) as a counterstain instead. Some bacteria which are poorly stained by safranin, such as *Haemophilus* spp., *Legionella* spp., and some anaerobic bacteria, are readily stained by basic fuchsin, but not safranin. The polychromatic nature of the gram stain enables determination of the size and shape of both Gram-negative and Gram-positive bacteria.

#### References:

1. Bartholomew, J. W. and Finkelstein, H., Relationship of cell wall staining to Gram differentiation, *J. Bacteriol.*, 75, 77, 1958.
2. Sydney M. Finegold and William J. Martin, *Diagnostic Microbiology*, Chapt. 3, Mosby Co., St. Louis, 1982.
3. Salton, M. R. J., *The Bacterial Cell Wall*, Elsevier Press, Amsterdam, 1964.
4. Gregersen, T., Rapid method for distinction of gram-negative from gram-positive bacteria, *Eur. J. Appl. Microbiol. Biotechnol.*, 5, 123, 1978.

