GRAM STAIN KIT

Cat.Nº.**4600**

PROTOCOL

1. Spread the culture with an inoculation loop to an even thin film over a circle of 1.5 cm diameter. The smear should be thin enough to dry completely within a few seconds.

2. Air-dry the culture and fix it or over a Bunser burner, 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. Heat can also be applied to facilitate drying the smear.

3. Add crystal violet stain over the fixed culture. Let stand for 1 minute. Pour off the stain and gently rinse the excess stain with a stream of water.

4. Add the Stabilized Lugol-PVP complex on the smear, enough to cover the fixed culture. Let stand for 1 minute. Pour off the iodine solution and rinse the slide with running water. Shake off the excess water from the surface.

5. Add a few drops of acetone-ethanol decolourant so the solution trickles down the slide. Rinse it off with water after 10 seconds. The exact time to stop is when the solvent is no longer colored as it flows over the slide. Further delay will cause excess decolorization in the gram-positive cells, and the purpose of staining will be defeated.

6. Counter stain with Safranine solution for 1 minute. Wash off the solution with water. The slide may be shaken to remove most of the water and air-dried.

7. A drop of immersion oil is placed on the stained bacterial smear. This helps transmit light through the specimen directly to the 100x microscope lens.

KIT COMPONENTS

Crystal violet oxalate solution	250 ml
Stabilized Lugol-PVP complex	250 ml
Decolorant (Acetone – Ethanol)	250 ml
Safranin solution	250 ml