

# Gram Stain Set (Stabilized)

Catalog Numbers: 25036

## DESCRIPTION

For staining bacteria from cultures or patient specimens by the differential Gram stain method. This procedure is based upon the Hucker modification of the original Gram stain method. Gram-positive and Gram-negative bacteria are both stained by the crystal violet stain. Addition of the iodine leads to the formation of a crystal violet-iodine complex within the cell wall. The decolorizer extracts lipids from the cell wall of Gram-negative bacteria, thereby increasing the porosity of the cell wall and allowing the crystal violet-iodine complex to diffuse from the cell. At the same time, Gram-positive bacteria are dehydrated, causing a decrease in cell wall porosity and trapping the crystal violet-iodine complex within the cell. Due to the increase in porosity, the safranin counterstain is able to permeate the cell wall of the Gram-negative bacteria.

**Kit Contains:** 6 Reagent Packs  
25036A Gram Stain Crystal Violet  
25036B Gram Stain Iodine  
25036C Gram Stain Decolorizer  
25036D Gram Stain Safranin  
Plastic Stand  
6 Reclosure Foils

## STORAGE CONDITIONS

Store at controlled room temperature (15-30°C) with bottles tightly sealed. Gram Stain Set reagent packs may be resealed with the included adhesive backed foil strip between uses. Do not use beyond the expiration date on the reagent pack.

## INDICATIONS OF DETERIORATION

If precipitate forms in the Crystal Violet Solution, warm gently to 37°C and shake well to re-dissolve.

## SPECIMEN COLLECTION & PREPARATION

**Liquid Media:** Spread the bacteria suspension on a clean glass slide using a sterilized wire loop and allow to air dry. Heat-fix the smear by passing the slide through a flame three times and allow to cool.

**Solid Media:** Place a loopful of deionized water on a clean glass slide. Touch a sterilized wire loop to a colony and suspend the cells in the water droplet; spread the suspension on the slide and allow to air dry. Heat-fix the smear by passing the slide through a flame three times and allow to cool.

**Patient:** Obtain the patient specimen using a sterile wire loop, pipette or swab. Smear the specimen on a clean glass slide and allow to air dry. Heat-fix the smear by passing the slide through a flame three times and allow to cool.

## TECHNIQUE

Immerse the prepared slide into chamber 1 (Crystal Violet Stain) for one minute. Rinse the slide gently with DI water. Immerse the slide into chamber 2 (Stabilized Iodine Solution) for approximately two minutes. Rinse the slide gently with DI water. Repeatedly dip the slide into chamber 3 (Decolorizer) inspecting between each dip. Decolorizing is complete as soon as solution runs clear from the smear on the slide. Rinse the slide gently with DI water. Immerse the slide into chamber 4 (Safranin) for one minute. Rinse the slide gently with DI water and blot the slide dry with absorbent paper. Examine the slide accordingly.

## RESULTS

Gram-positive bacteria are stained purple and Gram-negative are stained pink to red.

## LIMITATIONS OF PROCEDURE

Application of excessive heat during fixation of smear may rupture bacterial cell walls and affect the Gram staining results. Treatment with antibacterial drugs may cause Gram-positive bacteria to appear Gram-negative.

**Controls:** Performance of the Set may be tested using the following procedure: Stain a prepared slide of *Staphylococcus aureus* from an 18 to 24 hour culture with Crystal Violet Stain allowing to stand for one minute and rinsing with DI water. Gram positive purple cocci should be observed; if not, discard this solution.

Stain a prepared slide of *Escherichia coli* from an 18 to 24 hour culture with Safranin Stain allowing to stand for one minute and rising with DI water. Gram negative pink rods should be observed; if not, discard this solution.

Following the normal Gram staining procedure, stain a prepared slide of *S. aureus* from an 18 to 24 hour culture. Gram-positive purple cocci should be observed; if not, discard the Gram Iodine Solution.

## PRECAUTIONS

**Crystal Violet:** Causes eye irritation. Wash thoroughly after handling. In case of contact, flush eyes with water for 15 minutes. Contact a physician. See Safety Data Sheets for complete details.

**Iodine:** Causes eye irritation. Wash thoroughly after handling. In case of contact, flush eyes with water for 15 minutes. Contact a physician.

**Decolorizer:** Is extremely flammable. Use with adequate ventilation. Avoid excessive heat, sparks and open flame. Decolorizer causes eye irritation. Wash thoroughly after handling. In case of contact, flush eyes with water for 15 minutes. Contact a physician.

**Safranin:** Is a combustible liquid. Avoid excessive heat, sparks and open flame. Causes eye irritation. Wash thoroughly after handling. In case of contact, flush eyes with water for 15 minutes. Contact a physician.

## LIMITED WARRANTY

This product is warranted to perform as described in its labeling and product literature. Polysciences, Inc. disclaims all other warranties, expressed, implied and statutory with respect to this product, including, but not limited to the warranty of fitness for a particular purpose and the warranty of merchantability. In no event shall Polysciences, Inc. or any of its affiliates be liable for incidental or consequential damages of buyer or any third party, howsoever arising, even if advised of such damages.

## ORDERING INFORMATION

Cat. #	Description
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