



## GRAM STAIN SET

For staining bacteria from cultures or patient specimens by the differential Gram stain method

## SUMMARY

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.

## STORAGE CONDITIONS

Store at controlled room temperature (15-30°C) with bottles tightly sealed.

## INDICATIONS OF DETERIORATION

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

## SPECIMEN COLLECTION AND PREPARATION

**Liquid Media:** Spread the bacteria suspension on a clean glass slide using a sterile loop and allow to air dry. Heat-fix the smear by allowing smear to dry on a hot plate or in oven at ~42°C.

**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 allowing smear to dry on a hot plate or in oven at ~42°C.

**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 allowing smear to dry on a hot plate or in oven at ~42°C.

## GRAM IODINE PREPARATION

When following the traditional protocol, add the Gram Iodine concentrate to the Gram Iodine Diluent and mix well. Discard solution after three months from mixing.

## TECHNIQUE

1. Flood the prepared slide with Crystal Violet Stain and allow to stand for one minute.
2. Rinse the slide gently with DI water.
3. Flood the slide with Gram Iodine Working solution and allow to stand for one minute. (When using stabilized iodine, flood the slide with the solution and allow to stand for approximately two minutes).
4. Rinse the slide gently with DI water.
5. Gently rinse the slide with Decolorizer Solution for approximately ten seconds or until the decolorizer runs clear from the slide.
6. Rinse the slide gently with DI water.
7. Flood the slide with Safranin Stain and allow to stand for one minute.
8. Rinse the slide gently with DI water and blot the slide dry with absorbent paper.
9. Examine the slide.

## 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 rising 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 rinse 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 Working Solution.

## PRECAUTIONS

Highly flammable liquid and vapor. Harmful if swallowed. Causes skin irritation. Causes serious eye irritation. May cause drowsiness or dizziness. Causes damage to organs.

May cause long lasting harmful effects to aquatic life.

Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. Do not breathe dust/fume/gas/mist/vapors/spray. Wash exposed skin thoroughly after handling. Do not eat, drink or smoke when using this product. Wear protective gloves/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do so. Continue rinsing.

### Limited Warranty

This product is warranted to perform as described in its labeling and product literature as prepared. Astral Diagnostics, 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 Astral 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.

Cat. #	Description	Size
6060	Gram Stain Set, Stabilized Iodine	4x2oz
6250	Gram Stain Set, Traditional	4x8oz
6251	Gram Stain, Crystal Violet	4x8oz, 8oz, 16oz
6252	Gram Stain, Iodine Replacement, Traditional	4x8oz
6253	Gram Stain, Iodine, Stabilized	4x8oz, 8oz, 16oz
6254	Gram Stain, Decolorizer 75/25	4x8oz, 8oz, 16oz
6255	Gram Stain, Decolorizer Replacement 1:1	4x8oz
6256	Gram Stain, Safranin	4x8oz, 8oz, 16oz

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