

**GRAM'S STAINS KIT (CONTAINS BA087,BA088,BA027,BA028)**

**BA026**

**Formula**

**Reagents:**

**Gram's Crystal Violet (BA027)**

**Crystal Violet**

**Solution A:**

|                   |          |
|-------------------|----------|
| Crystal violet    | 2.00 g   |
| Ethyl alcohol 95% | 20.00 ml |

**Solution B**

|                  |           |
|------------------|-----------|
| Ammonium oxalate | 0.80 g    |
| Distilled water  | 80.00 ml. |

Solutions A and B mixed. Stored for 24 hours before use. The resulting stain is stable.

**Appearance:** Purple coloured solution

**Gram's Decolorizer (BA088)**

|                   |           |
|-------------------|-----------|
| Ethyl alcohol 95% | 50.00 ml  |
| Acetone           | 50.00 ml. |

**Appearance:** Colourless solution

**Gram's Iodine (BA087)**

|                  |           |
|------------------|-----------|
| Iodine           | 1.00 g    |
| Potassium iodide | 2.00 g    |
| Distilled water  | 300.00 ml |

**Appearance:** Yellow coloured solution

**Safranin 0.5% w/v (BA028)**

|                   |           |
|-------------------|-----------|
| Safranin O        | 0.50 g    |
| Ethyl alcohol 95% | 100.00 ml |

**Appearance:** Red coloured solution

**Counterstain working solution:**

Dilute 10 ml. of stock solution with distilled water to 100 ml.

**Directions:**

1. Prepare a thin smear on clear, dry glass slide.
2. Allow it to air dry and fix by gentle heat.
3. Flood with Gram's crystal violet(BA027) for 1 minute. (if over staining results in improper decolorization of known gram-negative microorganisms, use less crystal violet)
4. Wash with tap water.
5. Flood the smear with Gram's iodine (BA087). Allow to remain for 1 minute.
6. Flood smear with Gram's decolorizer (BA088) until the blue color no longer flows from the smear. (Acetone may be used as decolorizer with caution since this solvent rapidly decolorizes the smear).
7. Wash with tap water.
8. Counter stain with 0.5% w/v Safranin (BA028).Allow it to remain for 1 minute.
9. Wash with tap water.
10. Allow the slide to air dry or blot dry between sheets of clean bibulous paper and examine under oil immersion objective.

**Microscopy:**

|                              |               |
|------------------------------|---------------|
| Gram-positive microorganisms | - Violet      |
| Gram-negative microorganisms | - Pinkish red |

**Precautions :**

1. For Laboratory Use.
1. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

**Principle:**

Different mechanisms have been proposed to explain the gram reaction. There have been many physiological differences between gram-positive and gram-negative cell walls.

A thin smear of bacterial cells is stained with crystal violet, then treated with an iodine containing mordant to increase the binding of primary stain. A decolorizing solution of alcohol and/or acetone is used to remove crystal violet from cells which bind it weakly and then counterstain (e.g. safranin) is used to provide color contrast in those cells that are decolorised.

Gram-positive organisms' cells have more mucopeptide in their cell walls as compared to gram-negative ones. They later have more polysaccharides and lipoproteins in their cell walls. The polymer of glycerol or ribitol phosphate called teichoic acids are easily found in cell walls of gram-positive microorganisms but are not or significantly very less in those of gram-negative organisms.

**Storage:** Below 30°C

**Packing:** 100 ml each reagent

**Disclaimer:**

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related BIOMARK LABORATORIES publications.

The information contained in this publication is based on our in-house studies and market performance and is to the best of our knowledge true and accurate. BIOMARK LABORATORIES reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.