

BA026

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

Formula

Reagents:

Gram's Crystal Violet (BA027)

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: Light brown 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.

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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

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 decolorising 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 also have more polysaccharides and lipoproteins in their cell walls. The polymer of glycerol or ribitol phosphate called teichoic acids are also found in cell walls of gram-positive microorganisms but are not or significantly fewer in those of gram-negative organisms.

Storage: Below 30°C

Packing: 100 ml each reagent

Warning & Precautions :

In Vitro diagnostic Use only. For professional use only. For Laboratory Use. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets

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Use:

Gram's staining of microbes.

Disposal:

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

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 BIOMARKLABORATORIES publications.

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