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 2.5% w/v (BA028)

Safranine O	2.50 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 0.25% 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	- Bluish purple
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.Ther 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 trated with an iodine containing mordant to increase the binding of primary stain.A doclorising solution of alcohol and/or acetone is used to remove crystal vilet 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.he later have more polysaccharides and lipoproteins in their cell walls. The polymer sof glycerol or ribitol phosphate called teichoic acids ar easlo found in cell walls of gram-positive microoganisms but at not or significantly very lee in those of gram-negative organisms.

Storage: Below 30°C.

Packing: 100 ml each reagent