Gram Staining Kit RDM-K-GS-01

Principle

The cell walls for Gram-positive microorganisms have a higher peptidoglycan and lower lipid content than Gram-negative bacteria. The cell walls of gram-positive bacteria have a thick layer of protein-sugar complexes called peptidoglycan and lipid content is low. Gram staining is based on the ability of bacterial cell wall to retain the crystal violet dye during solvent treatment. When the bacterial cell are stained with primary stain Crystal Violet and fixed by the mordant, some of the bacteria are able to retain the primary stain and some are decolorized by alcohol. Decolorizing the cell causes this thick cell wall to dehydrate and shrink which closes the pores in the cell wall and prevents the stain from exiting the cell. So the ethanol cannot remove the Crystal Violet-Iodine complex that is bound to the thick layer of peptidoglycan of gram positive bacteria and appears blue or purple in colour. In case of gram-negative bacteria, cell wall also takes up the CV-Iodine complex but due to the thin layer of peptidoglycan and thick outer layer which is formed of lipids, CV-Iodine complex gets washed off. When they are exposed to alcohol, decolorizer dissolves the lipids in the cell walls, which allows the crystal violet-iodine complex to leach out of the cells. Then when again stained with safranin, they take the stain and appear red in color.

Use: To differentiate between Gram-positive and Gram-negative bacteria.

Contents*

Preparation	**Ingredients**	**Concentration**
Crystal violet solution	Ethanol	8.00 % v/v
	Crystal Violet	< 1.00 w/v
Decolorizer solution	Acetone	40.00 % v/v
	Ethanol	57.00 % v/v
Iodine Solution	Iodine	0.33 % w/v
Safranine solution	Ethanol	2.00 % v/v
	Safranine	< 1.00 % w/v

* Formula adjusted for optimum performance and parameters

Directions:

1. Make a smear on grease free clean slide, heat fix it.
2. Flood 1 drop of Crystal violet on the smear for 1 min; wash with d/w.
3. Flood with 1 drop of Gram`s iodine the smear for 1 min; wash with d/w.
4. Flood with de-colorizer drop wise, until the smear oozes the stain.
5. Flood with 1 drop of Safranine stain the smear for 1 min; wash with d/w.
6. Allow to air dry, and observe under microscope.
7. Purple colored organisms are Gram Positive, while Pink colored organisms are Gram negative.

Specimens' types analyzed

Bacterial samples etc.

Precautions to be taken

1. Reagents must be added in the order and the amounts specified or a weak-positive or false-negative

reaction may occur.
2. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.
3. The test is an aid to identification and is not a confirmatory test. Complete identification should include determination of Gram reaction, morphology, and other biochemical and serological tests.
4. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.

Performance and Evaluation

The expected performance of the stain kit is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

Physical Parameters:

Physical state - Liquid.
Crystal Violet - Violet.
Decolorizer - Colorless.
Iodine - Yellowish-Brown.
Safranin - Red.

Different Microbial Response

Organism	ATCC	Cell morphology	Gram`s character
Staphylococcus aureus	25923	Cocci	Gram positive
Bacillus spizizenii	6633	Rod shape	Gram positive
Pseudomonas aeruginosa	27853	Rod shape	Gram negative
Salmonella typhi	6539	Rod shape	Gram negative
Escherichia coli	8739	Rod shape	Gram negative

Storage and Shelf Life: Keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label.

Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. Atlas, R. M. (2005). *Handbook of media for environmental microbiology*. CRC press.
2. Bartholomew, J. W. (1962). *Variables influencing results, and the precise definition of steps in gram staining as a means of standardizing the results obtained.* Stain Technol. 37:139-155.
3. *Difco Manual* (1998). 11th Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.
4. Murray, P. R. (ed.). (1995). *Manual of Clinical Microbiology*, 6th ed. American Society of Microbiology, Washington, D.C.

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