

## Product Manual

### 【Product Name】

Gram Stain

### 【Product Code and Packing Specification】

Code	Type	Specifications
029010	Liquid	10mL ×4 vial

### 【Product Usage】

For bacterial Gram staining experiments

### 【Test Principle】

After primary staining with crystal violet and mordanting with iodine solution, a water-insoluble complex of crystal violet and iodine is formed in the cell wall. Gram-positive bacteria have thicker cell walls, more layers of peptidoglycan network and dense cross-linking. Therefore, when they are decolorized with ethanol or acetone, the mesh holes will shrink due to water loss. In addition, they do not contain lipids, so there will be no gaps after ethanol treatment. Therefore, the crystal violet and iodine complex can be firmly retained in the wall, making it still purple. Gram-negative bacteria have thin cell walls, high lipid content in the outer membrane, thin peptidoglycan layer and poor cross-linking. After encountering the decolorizer, the outer membrane mainly composed of lipids dissolves rapidly, and the thin and loose peptidoglycan network cannot prevent the dissolution of the crystal violet and iodine complex. Therefore, they are still colorless after decolorization with ethanol, and then re-stained with red dyes such as safflower, which makes Gram-negative bacteria red.

### 【Formulation】

#### 1. Crystal violet staining solution

Formulation (per vial)	Content
Crystal violet	0.1g
95% ethanol	2mL
1% ammonium oxalate aqueous solution	8mL

#### 2. Gram's iodine solution

Formulation (per vial)	Content
Iodine	0.033g
Potassium iodide	0.067g
Distilled water	10mL

#### 3. Dehydrated alcohol

Formulation (per vial)	Content
95% ethanol	10mL

#### 4. Sand yellow counterstain

Formulation (per vial)	Content
Sand yellow	0.025g
95% ethanol	1mL
Distilled water	9mL

### 【Instructions】

1. Fix the smear on the flame, add crystal violet staining solution. Dye for 1min, wash with water.
2. Use an empty bottle to hold the required Gram iodine solution, add Gram iodine solution before use, and let it act for 1min. Wash with water. The remaining iodine solution should be promptly put back into the brown glass bottle.
3. Add decolorizing alcohol for about 30s; or drip decolorizing alcohol all over the smear, pour it off immediately, and then drip 95% alcohol all over the smear, decolorize for 10s.
4. Wash with water, add safranin counterstaining solution, counterstain for 1min, wash with water, wait to dry, and examine under a microscope.
5. Results: Gram-positive bacteria are purple, and Gram-negative bacteria are red.

**【Quality Control】**

Index	Quality control strain and number	特征性反应
Feature observation	<i>Escherichia coli</i> ATCC25922	Gram-negative, red under the microscope, short rod-shaped Gram-positive, purple under the microscope, spherical
	<i>Staphylococcus aureus</i> ATCC6538	Gram-negative, red under the microscope, short rod-shaped Gram-positive, purple under the microscope, spherical

**【Storage Conditions and Shelf Life】**

Store at room temperature away from light, valid for three years.

**【Waste Disposal】**

After testing, the contaminated items were sterilized by autoclaving at 121°C for 30 minutes.

**【Executive Standard】**

Q/HKSJ 0709 Guangdong Huankai Microbial Sci.& Tech.Co.,Ltd. Enterprise Standard

**【Explanatory Version】**

May 14, 2019

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