

PRODUCT SPECIFICATION SHEET



Safranin-0.5% w/v(SI028)

Use

Safranin is used for Gram's staining.

Principle

Gram staining basically involve six basic steps:

1. Preparation of smear
2. Fixing of smear by heating
3. Staining with crystal violet
4. Use of mordant (iodine)
5. Treatment with alcohol-acetone for decolourisation
6. Counter staining with safranin

The air-dried, fixed smear of bacteria, when treated with crystal violet stain (Gram stain) and the mordant (iodine), picks up the stain, and looks purple. Iodine, which acts as a mordant, enhances the union between the dye and the internal contents of the organism. The subsequent step, alcohol-acetone treatment, takes away the colour and decolorizes some bacteria, which are designated as gram negative. On the other hand, gram positive bacteria will retain the colour. Following decolourization, counterstaining with safranin stains the gram negative bacteria red while the gram positive bacteria retain the original crystal violet stain (Gram stain). When viewed under the microscope the gram negative organisms are red in colour and the gram positive organisms are purple in colour.

Formulation

Sr No.	Items/Ingredients	Grams/Litre
1.	Safranin powder	5
2.	Ethyl Alcohol	1000

Storage And Stability

1. Store the bottle in dry, cool and dark place.
2. The shelf life of reagents is as per the expiry date mentioned on the reagent bottle labels.

Procedure

1. Transfer the specimen to a clean microscope slide. Prepare a smear and air dry.
2. Heat-fix the slide by passing through the flame and place on a staining rack with smear side up.
3. Flood the slide with **Gram Crystal Violet** (SI010) for 1 minute.
4. Rinse with tap water and flood with **Gram Iodine** (SI016).
5. Wait for 1 minute and rinse with water.
6. Decolorize with **Gram Decolorizer** (SI 002) until no more dye is released. Immediately rinse with tap water.
7. Flood the slide with **Gram Safranin** (SI028) for 30 seconds and rinse.
8. Wipe any stain that remains off the back of the slide and blot dry or air dry completely.



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PRECAUTIONS

1. For In-vitro Diagnostic use only
2. Overheating of organisms during fixing process may disintegrate organisms on the smear.
3. Decolourising process timing depends upon the thickness of smear and adequate allowance should be given depending upon the tube of the specimen.
4. Product to be used only by adequately trained and qualified laboratory personnel.

QUALITY CONTROL

Appearance: Red Colour solution

Microscopic Examination

Using Crystal Violet as a stain, gram staining is carried out and staining characteristics of microorganisms are observed under microscope by using oil immersion lens.

Run controls using 18-24 hour standard ATCC cultures of known gram-positive and gram-negative microorganisms. The following test strains are recommended:

Organism	Expected Results	
<i>Staphylococcus aureus</i> ATCC 25923	Gram-positive cocci	Dark purple colored
<i>Escherichia coli</i> ATCC 25922	Gram-negative rods	Red or pink colored

Packaging

Product Name : **Safranin**

Product Code : **SI028**

Available Pack sizes : **500ml/125ml**

Further Information

For further information please contact your local MICROMASTER Representative.



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