

# Safranin Solution

Cat. 5063

Colouring, fixating and decolouring solutions for bacterial classification according to gram stain.

## Practical information

Applications	Categories
Differentiation	Gram-positive bacteria
Differentiation	Gram-negative bacteria

Industry: Dyes and stains

## Principles and uses

The Gram stain procedure differentiates microorganisms into two groups, those which retain the primary dye (Gram-positive) and those which lose the primary dye, due to the structure of cellular wall, and take the colour of the counterstain (Gram-negatives).

The procedure needs four reagents: Primary dye (Oxalate Crystal Violet Solution), Iodine solution (Lugol), Decolorizer (Acetone Ethanol Decolorant) and Counter stain (Safranin Solution).

## Formula in g/L

Ethanol	200	Safranin	4,5
Water	795,5		

## Instructions for use

Prepare a smear and heat-fix it by gentle heating in the flame.

- 1- Cover the smear with Crystal Violet. Let stand for 1 min.
- 2- Remove excess by rinsing with tap water.
- 3- Cover with Lugol and allow standing for 1 min.
- 4- Decant and rinse with tap water.
- 5- Decolorize with Acetone Ethanol Decolorant until waste decolorizer were colourless.
- 6- Rinse with tap water.
- 7- Counter stain with Safranin Solution for 1 min.
- 8- Rinse with tap water and air dry.

Examine under an oil immersion objective.

The procedure can be modified according to the user's preferences to achieve a weaker or stronger colour intensity, being carried out by changing the times for staining, washing etc.

Old cultures or smears could give atypical results. That is why cultures of 18-24 hours or recent smears are recommended.

It is very important to control the heat-fixation (few seconds), any excess heating could produce erroneous results. Highly chlorinated tap water could weak the counter staining.

## Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Liquid	N/A	N/A	N/A

## Microbiological test

Microrganisms	Specification
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Gram-positive bacteria  
Gram-negative bacteria

Blue-purple colonies  
Pink-red colonies

## Storage

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Temp. Min.:15 °C  
Temp. Max.:30 °C

## Bibliography

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Clark, G. (1981) "Staining Procedures", 4th ed, Williams&Willkins.  
Bartholomew J.M., Mitwer, T. (1952), Bacteriol. Rev., 16, 1-29.