

Gram Stain

Cat. 4600

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 its cellular wall, and take the colour of the counterstain (Gram-negatives).

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

Formula in g/L

Acetone Ethanol Decolorant			
Ethanol	970	Acetone	30
Lugol			
Water	990	Iodine	3,5
Potassium iodide	6,5		
Oxalate Crystal Violet Solution			
Ethanol	200	Water	787
Ammonium oxalate	7,5	Crystal violet	5,5
Safranin Solution			
Safranin	4,5	Water	795,5
Ethanol	200		

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
Gram-positive bacteria	Dark violet colonies
Gram-negative bacteria	Orange-red colonies

Storage

Temp. Min.:15 °C
Temp. Max.:30 °C

Bibliography

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