

## Lugol

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

### Practical information

Applications	Categories
Detection	Gram-positive bacteria
Detection	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

Iodine	4	Potassium iodide	7
Water	989		

### 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	Appareance	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

Note: Any interference is not known. Acid, basic or high levels of Chloride or salts in wash water could alter the results.

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## Microrganisms

Gram-positive bacteria  
Gram-negative bacteria

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## Characteristic reaction

Blue to purple colonies  
Pink to red colonies

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## Storage

Temp. Min.:15 °C  
Temp. Max.:30 °C

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## Bibliography

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