

# Acetone Ethanol Decolorant

Cat. 4614

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

|         |     |         |     |
|---------|-----|---------|-----|
| Acetone | 300 | Ethanol | 700 |
|---------|-----|---------|-----|

## 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                          | 3,5 - 7,3       |

## Microbiological test

| Microrganisms          | Specification        |
|------------------------|----------------------|
| Gram-positive bacteria | Blue-purple colonies |

Gram-negative bacteria

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.