



Neutral Red Solution for Gram Staining

Introduction

The Gram staining method is a classic technique used to differentiate between Gram-positive and Gram-negative bacteria based on the characteristics of their cell walls. While the traditional Gram stain uses crystal violet and safranin, neutral red can be employed in some protocols to enhance visualization, particularly in the context of specific experiments.

Components and Recommended Storage

Component	Catalog No.	Format	Storage
Neutral red solution 0.33 g/L in ultra-pure water	115-341-061	100 ml	4-8°C

Materials

- Crystal violet [primary stain]
- Iodine solution/Gram's Iodine (mordant that fixes crystal violet to the cell wall)
- Decolorizer (*e.g.* ethanol or acetone)
- Neutral red solution (**Quality Biological #115-341-061**) [secondary stain]
- Ultrapure water (**Quality Biological #351-029-101**).

Protocol

1. Preparation of Bacterial Smear:

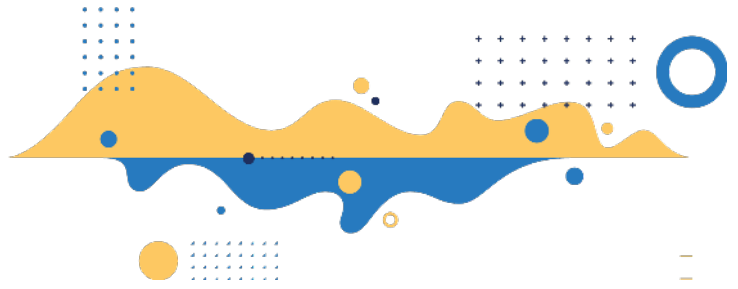
- Using a sterile inoculating loop, place a small amount of the bacterial culture onto a glass slide.
- Spread the culture to create a thin smear.
- Allow the smear to air dry completely.
- Heat-fix the smear by passing the slide through a flame a few times.

2. Staining with Crystal Violet (primary stain):

- Flood the smear with crystal violet solution and let it sit for about 1 minute.
- Rinse the slide gently with ultrapure water.

3. Mordant with Iodine:

- Apply Gram's iodine to the smear for 1 minute.
- Rinse gently with distilled water. The iodine is a mordant, or an agent that fixes the crystal violet to the



bacterial cell wall.

4. Decolorization:

- Apply the decolorizer (ethanol or acetone) dropwise for about 10-30 seconds, or until the wash runs clear. This step is crucial as it differentiates the two types of bacteria:
 - Gram-positive bacteria retain the crystal violet-iodine complex and remain purple.
 - Gram-negative bacteria lose the color and become colorless.

5. Staining with Neutral Red (secondary stain):

- Flood the smear with neutral red solution and let it sit for about 1-2 minutes.
- Rinse gently with ultrapure water. Neutral red stains the decolorized Gram-negative bacteria pink.

6. Microscopic Examination:

- Allow the slide to air dry.
- Observe under a microscope.
- Gram-positive bacteria should appear purple, while Gram-negative bacteria will appear pink.

If the bacteria is Gram positive, it will retain the primary stain (crystal violet) and not take the secondary stain (neutral red), causing it to look violet/purple under a microscope. If the bacteria is Gram negative, it will lose the primary stain and take the secondary stain, causing it to appear reddish-pink when observed under a microscope.

Notes

- Always wear appropriate personal protective equipment including lab coat, gloves, and safety glasses when handling the neutral red solution.
- This protocol consists of general guidelines to perform bacterial gram staining; it may be necessary to optimize conditions, such as the time of primary and secondary staining according to the experimental goals.
- It is recommended to perform preliminary testing to determine the optimal staining time.
- After extended storage of the neutral red solution, a precipitate might occur in the form of small particles. If the neutral red solution contains precipitates, use the supernatant only, or filter the solution to remove the precipitates.
- The Quality Biological neutral red solution is for research or further manufacturing use only, not for diagnostics or therapeutic use.