Crystal Violet Ammonium Oxalate Solution (2%)

A1506562

Storage: Room Temperature. Store in the dark.

Introduction:

The molecular formula of ammonium oxalate is $C_2H_{10}N_2O_5$ with a molecular weight of 142.11, while the molecular formula of crystal violet is $C_{25}H_{30}ClN_3$ with a molecular weight of 407.99. Crystal Violet Staining Solution is a commonly used staining reagent for tissue or cell samples, which stains cell nuclei dark purple. It is primarily applied in Gram staining. As a basic dye, crystal violet can bind to DNA in the cell nucleus, thereby achieving nuclear staining.

Aladdin Crystal Violet Ammonium Oxalate Solution is a key component of the Gram staining method, consisting mainly of ammonium oxalate, crystal violet, and ethanol. In the staining of Gram-positive bacteria, the cells are first stained with ammonium oxalate crystal violet and then treated with Gram's iodine solution, forming an insoluble complex. This complex cannot penetrate the bacterial cell wall and is not easily decolorized, thus retaining the purple color. The staining exhibits a color transition from green (at pH 0.5) to blue (at pH 2.0). After staining, bacteria form a sharp contrast against the background, allowing for clear observation of their morphology, arrangement, and certain structural characteristics, which facilitates bacterial classification and identification.

Materials to be prepared by the user: Inoculation loops, other tools for picking bacteria, alcohol lamps, glass slides, and light microscopes.

Operating Procedures (For Reference Only):

1. Operate according to the specific requirements of the experiment or refer to the following Gram staining method.
2. Smear Preparation: Take the bacteria to be tested and spread them into a thin layer at the center of a glass slide. Alternatively, place a drop of sterile water on the glass slide, mix the bacterial suspension thoroughly with the water, and then spread it into a thin layer.
3. Drying: Air-dry the smear at room temperature naturally. You may also slightly heat the slide over an alcohol lamp to accelerate drying.
4. Fixation: Hold one end of the glass slide with the specimen side facing up, and move it quickly back and forth 3–5 times over the outer flame of an alcohol lamp (1 second per pass). Avoid excessive temperature to prevent denaturation of bacterial proteins. Allow the slide to cool before staining. Methanol or ethanol can also be used for fixation.
5. Primary Staining: Cover the specimen with crystal violet staining solution and stain for 1–2 minutes. Rinse the slide with clean water to remove the staining solution.
6. Mordanting: Add Gram's iodine solution to cover the entire glass slide, leave it at room temperature for 1–2 minutes, and then rinse with water.
7. Decolorization: Apply decolorizing solution and shake the slide continuously for 10–30

seconds until no purple color runs off with the decolorizing solution. Immediately rinse the slide with water to terminate the reaction.

8. Counterstaining: Stain the slide with safranin staining solution for 30–60 seconds, followed by a water rinse.
9. Drying and Microscopic Examination: Dry the slide and observe under an oil immersion lens.

Staining Results:

Gram-positive bacteria	Blue to purple
Gram-negative bacteria	Red

Precautions:

1. Before preparing the smear, mark a circle on the back of the glass slide in advance to determine the position for subsequent tests.
2. Pay attention to personal protection when collecting bacteria. When removing the test tube stopper, pass the mouth of the test tube through an alcohol lamp flame for slight flaming sterilization. Finally, sterilize the inoculation loop by flaming it over the alcohol lamp.
3. When heat-fixing the smear, make sure the glass slide is not too close to the flame. Generally, the temperature of the slide should not exceed 60°C, which can be judged by touching the back of the slide to the back of your hand— it should not feel excessively hot.
4. The key to Gram staining is strictly controlling the decolorization degree, and the decolorization time should be determined based on experience. Over-decolorization may cause Gram-positive bacteria to be mistakenly stained as Gram-negative; insufficient decolorization may lead to Gram-negative bacteria being misidentified as Gram-positive.
5. The culture time of the bacteria to be tested will affect the staining result. Gram-positive bacteria that have been cultured for an excessively long time, or are already dead or lysed, often show a Gram-negative staining pattern.
6. For your safety and health, wear a lab coat and disposable gloves during the operation.