Standard Gram Staining Solution

Cat #: A-CSH312

Size: 4×10mL, 4×100mL, 4×250mL, 4×500mL

Storage: Room temperature, avoid light (12 months)

Product Composition

Component	4×100mL	4×500mL	Store at
Reagent (A): crystal violet staining solution	100ml	500ml	10~30°C, and were protected from light
Reagent (B): Gram iodine solution	100ml	500ml	10~30°C, and were protected from light
Reagent (C): Decolorization solution	100ml	500ml	10~30°C
Reagent (D): yellow staining solution	100ml	500ml	10~30°C, and were protected from light

Product Description

The Gram staining method is a widely used differential staining technique in bacteriology and also a counterstain method. Unstained bacteria are difficult to observe under a microscope because their refractive index is very similar to the surrounding environment. After staining, bacteria form a distinct contrast with the environment, allowing for clear observation of their morphology, arrangement, and certain structural features for classification and identification. This staining method can differentiate bacteria into two major categories: Gram-positive (G+) and Gram-negative (G-) bacteria. The different staining reactions of bacteria are due to the differences in cell wall permeability to ethanol and resistance to decolorization, primarily determined by the thickness and structure of the peptidoglycan layer. Cells stained with crystal violet form insoluble complexes after treatment with iodine solution, which can be decolorized by ethanol. In Gram-negative cell staining, ethanol or acetone disrupts the outer membrane, damages the peptidoglycan layer and cytoplasmic membrane, causing leakage of crystal violet and iodine complexes from the cells. When re-stained with other staining solutions, they appear red. Although red dye can also enter Gram-positive (G+) cells that have been stained purple, it is masked by the purple color, so the red color is not visible. In Gram-positive cell staining,

ethanol also dehydrates the thick peptidoglycan layer, reducing pore size. Due to the large size of crystal violet and iodine complexes, they cannot pass through the cell wall easily, resulting in the retention of the purple color.

The standard Gram staining solution uses the most classic Gram staining formula. Direct smearing of clinical specimens results in a clean background, strong contrast between the nucleus and cytoplasm, clear identification of intracellular inclusion bodies, and typical bacterial staining characteristics..

Usage

1. Smearing: Take the bacteria to be tested and spread them in a thin layer in the center of a glass slide or add a few drops of sterile water onto the slide. Mix the bacteria and water evenly, spreading them into a thin layer.

2. Drying: Allow the slide to air dry at room temperature or gently heat it over an alcohol lamp to expedite drying.

3. Fixation: Hold one end of the glass slide, with the specimen side facing up, and quickly pass it back and forth outside the flame of an alcohol lamp 3-5 times, 1 second each time. The temperature should not be too high to prevent denaturation of bacterial proteins. Let it cool before staining. Alternatively, fixation can be done using methanol or ethanol.

4. Primary Staining: Add crystal violet staining solution and let it stain for 1-2 minutes. Rinse off the staining solution with water.

5. Mordanting: Add Gram's iodine solution, cover the glass slide, and let it sit at room temperature for 1-2 minutes. Rinse with water.

6. Decolorization: Add the decolorizing solution and shake gently for 10-30 seconds, or until the decolorizing solution no longer appears purple when it drips off. Immediately rinse off the decolorizing solution with water to stop the reaction.

7. Counterstaining: Add safranin staining solution and let it stain for 30-60 seconds. Rinse with water.

8. Drying and Microscopic Examination: Use an oil immersion lens for observation.

Staining Results

gram-positive bacterium	blueTo purple
gram-negative bacterium	red

Notes

1. Before smearing, it is recommended to make circular markings on the back of the slide to determine the position for subsequent experiments.

2. When handling bacteria, personal protection should be taken into consideration. When removing the test tube cap, briefly pass the tube mouth through a flame for sterilization. Finally, sterilize the inoculation loop by flaming it in the flame.

3. When heat-fixing the smear, be cautious not to hold the slide too close to the flame. Generally, the slide temperature should not exceed 60°C, and it should feel comfortably warm when touched on the back of the hand.

4. The key to Gram staining lies in controlling the decolorization process strictly. The decolorization time should be determined based on experience. Over-decolorization can lead to false staining of Gram-positive bacteria as Gram-negative, while insufficient decolorization can result in false staining of Gram-negative bacteria as Gram-positive.

5. The culture time of the bacteria to be tested can affect the staining results. Prolonged culture time, bacterial death, or bacterial dissolution often lead to a negative staining appearance.

6. For your safety and health, please wear a lab coat and disposable gloves while conducting the experiment.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.