Enhanced Gram Staining Solution

Cat #: A-CSH313

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): compound 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 due to their minimal difference in refractive index with the surrounding environment. After staining, bacteria show 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. Crystal violet-stained cells form insoluble complexes when treated 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 the leakage of crystal violet and iodine complexes from the cells. When restained with another staining solution, they appear red. Although the red dye can also enter the already purple-stained G+ cells, it is overshadowed by the purple color, so the red color does not show. 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 complex molecules, they cannot pass through the cell wall, resulting in difficulty in decolorization, thus retaining the purple color.

Enhanced Gram staining solution further improves the classic Gram staining formula by using a counter red staining solution instead of safranin staining solution, enhancing the staining efficiency for difficult-to-stain bacteria. Direct smears of clinical specimens show a clean background, strong contrast between nuclei and cytoplasm, and clear recognition of intracellular inclusions, with typical bacterial staining characteristics.

Usage

1. Smear: Take the bacteria to be tested and spread them evenly in a thin layer in the center of a glass slide or add a small amount of sterile water to the glass slide, mix the bacteria with water evenly, and spread them into a thin layer.
2. Drying: Allow the smear to air dry at room temperature or gently heat it over an alcohol lamp to facilitate rapid drying.
3. Fixation: Hold one end of the glass slide and quickly move it back and forth outside the flame of an alcohol lamp for 3-5 times, 1 second each time. The temperature should not be too high to prevent denaturation of bacterial proteins. Let it cool down before staining. Alternatively, fixation can be done with methanol or ethanol.
4. Primary staining: Add crystal violet staining solution and stain for 1-2 minutes, then rinse off the staining solution with water.
5. Mordant: Add Gram's iodine solution, cover the glass slide, and let it sit at room temperature for 1-2 minutes, then rinse with water.
6. Decolorization: Add the decolorizing solution and shake for 10-30 seconds, until the flowing decolorizing solution no longer appears purple. Immediately rinse off the decolorizing solution with water to stop the reaction.
7. Counterstaining: Add the counter red staining solution and stain for 30-60 seconds, then rinse with water.
8. Drying and microscopic examination: Place the slide under an oil immersion lens for observation

Staining Results

gram-positive bacterium	blueTo purple
gram-negative bacterium	red

Notes

1. Before smearing, it is advisable to make circular markings on the backside of the slide to facilitate the identification of the subsequent experiments' locations.

2. When obtaining bacteria, self-protection should be taken into consideration. When removing the test tube cap, the tube opening should be briefly scorched by a flame. Finally, the inoculating loop should be sterilized by flaming it in the flame.

3. When heating to fix the smear, caution should be exercised to keep the slide at a moderate distance from the flame. Generally, the slide temperature should not exceed 60°C, and it should be deemed appropriate if the back of the slide touches the skin without feeling too hot.

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

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

Disclaimer

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