

Safranin Counterstain Solution

Cat #: A-CSH386

Size: 100mL

Storage: Room temperature, avoid light (12 months)

Product Composition

Component	Size	Store at
Shahuang redye liquid	100ml	10~30°C, and were protected from light

Product Description

The Gram staining method was invented by Danish physician Christian Gram in 1884. It is a widely used differential staining method in bacteriology and also a type of counterstaining. Unstained bacteria are difficult to observe under a microscope due to their minimal difference in refractive index compared to 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, which aids in classification and identification.

The Safranin counterstain solution is mainly composed of safranin and is commonly used in combination with crystal violet and iodine solution to detect Gram-positive and Gram-negative bacteria.

Usage

1. Smear Preparation: Take the bacteria to be tested and spread a thin layer in the center of a glass slide or add a few drops of sterile water onto the slide, mix the bacteria with the 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 expedite the drying process.
3. Fixation: Hold one end of the slide and pass the specimen side quickly 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 let it stain for 1-2 minutes, then rinse off the staining solution with water.

5. Mordant: Add Gram's iodine solution, cover the slide, and leave it at room temperature for 1-2 minutes, then rinse with water.

6. Decolorization: Add the decolorizer, shake gently for 10-30 seconds, until the runoff no longer appears purple, immediately rinse off the decolorizer with water to stop the reaction.

7. Counterstaining: Add the safranin staining solution and let it stain for 30-60 seconds, then rinse with water.

8. Place a clean coverslip over the slide and gently press to make a smear.

Staining Results

gram-positive bacterium	Blue to purple
gram-negative bacterium	red

Notes

1. Prior to smearing, it is advisable to mark a circle on the back of the slide to determine the location for subsequent tests.

2. The culture time of the bacteria to be tested can affect the staining results. Prolonged culture time of Gram-positive bacteria, bacterial death, or bacterial dissolution often result in Gram-negative staining.

Disclaimer

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