

Gram Staining Protocol

Gram staining

1. Make a slide of tissue or body fluid that is to be stained. Heat the slide for few seconds until it becomes hot to the touch so that bacteria are firmly mounted to the slide.
2. Add the primary stain crystal violet and incubate 1 minute.
3. Add Gram's iodine for 1 min. It is not a stain; it is a mordant. It doesn't give color directly to the bacteria but it fixes the crystal violet to the bacterial cell wall.
4. Wash with Decolorizer. If the bacteria are Gram-positive it will retain the primary stain. If it is Gram-negative it will lose the primary stain.
5. Add the secondary stain, safranin, and incubate 1 min, then wash with water for a maximum of 5 seconds. If the bacteria are Gram-positive then it will retain the primary stain and will not take the secondary stain. It will look black-violet in a pink background. If it is Gram-negative then it will lose the primary stain and take secondary stain making it pink-red.

Gram Stain is 2 g of 90% crystal violet dissolved in 20 ml of 95% ethyl alcohol.

Gram's iodine is 1 g of iodine, 2 g of potassium iodide, dissolved in 300 ml of distilled water.

Decolorizer is 50% ethyl alcohol, 50% acetone.[1]