

Gram Staining (Hucker Modification)

Reagents Needed:

Gentian Violet Stain, Hucker Formula	RICCA CHEMICAL COMPANY Cat. No. 3240
Gram's Iodine Stain	RICCA CHEMICAL COMPANY Cat. No. 3450
or Lugol's Iodine	RICCA CHEMICAL COMPANY Cat. No. 4440
Acetone-Alcohol, 1 + 1 Decolorizer Solution	RICCA CHEMICAL COMPANY Cat. No. 200
Safranin Counterstain	RICCA CHEMICAL COMPANY Cat. No. 6680

Recommended Method:

1. Use a clean glass slide that has been stored in 70% Ethanol. Remove with forceps and flame-sterilize. Identify and divide the slide, if necessary, using a wax pencil.
2. Transfer a portion of the culture using a flame-sterilized loop. (For liquid media, shake well before sampling. For solid media, emulsify a segment of colony in a loopful of sterile water.) Spread over an area of about 1 cm. Use thick smears for liquid media and thin smears of emulsified colonies taken from solid media.
3. Allow to air dry.
4. Fix by quickly passing the slide, smear side up, five or six times through the top of a Bunsen burner flame.
5. Allow slide to cool to room temperature.
6. Flood the slide for 1 minute with Gentian Violet Stain, Hucker Formula (RICCA CHEMICAL COMPANY Cat. No. 3240).
7. Wash with tap water just long enough to remove the excess stain. If the slide is placed directly in the stream of tap water, wash for 1 to 2 seconds. If the slide is placed in a 250 mL beaker into which tap water is running at the rate of about 30 mL/second, wash for about 5 seconds.
 Caution: This is a critical step. The degree of washing at this point will affect acceptable decolorization time.
8. Flood the slide with Gram's Iodine Stain (RICCA CHEMICAL COMPANY Cat. No. 3450), drain, and flood again for 1 minute with Gram's Iodine Stain.
 Note: Gram's Iodine can also be prepared by diluting one volume of Lugol's Iodine (RICCA CHEMICAL COMPANY Cat. No. 4440) with 14 volumes of purified water.
9. Wash the slide with tap water for about 5 seconds. Do not blot or dry.
10. Decolorize with Acetone-Alcohol, 1 + 1 Decolorizer Solution (RICCA CHEMICAL COMPANY Cat. No. 200) until no further violet color washes off the slide. Allow the decolorizer to run over the surface of the slide at a constant slow rate. Decolorization times may vary: usually 5-15 seconds for thin smears and 15-60 seconds for thick smears.
 Caution: Overdecolorization may result in loss of differentiation between Gram-Positive and Gram-Negative microorganisms.
11. Wash thoroughly with high purity water to stop the action of the decolorizer.
12. Flood the slide with Safranin Counterstain (RICCA CHEMICAL COMPANY Cat. No. 6680) for about 30-60 seconds.
13. Wash very lightly with tap water.
 Caution: Overwashing will remove the Safranin Counterstain from Gram-Negative microorganisms, causing them to appear small and lightly stained.
14. Blot dry, then air dry, and examine.

Because of the danger of overstaining or overdecolorization, it is recommended that controls of known Gram-Positive and Gram-Negative microorganisms be stained along with the cultures being examined at least once a day.

Satisfactory Staining Results:

Gram-Positive microorganisms stain dark Blue, Blue-Black, or Purple.

Gram-Negative microorganisms stain Red.

This is a typical staining procedure. These reagents may be suitable for other staining procedures. Consult staining reference books or standard operating procedures for other suitable uses of these products.