



GRAM STAIN REAGENT PREPARATION

Procedural note: It is highly recommended that gloves, lab coats, and eye protection be worn while preparing these stains. The dried components and the formulated stains soil anything they come into contact with.

Crystal Violet:

Prepare the two solutions in separate flasks containing stirring bars:

- A. Solution A: 2.0 gm. Crystal Violet is dissolved in a solution of 20.0 ml 95% Ethanol and 80.0 ml Deionized water.
- B. Solution B 4.0 gm. of Ammonium Oxalate is dissolved in 400.0 ml of Deionized water. This may require slight heating while stirring.

Mix both solutions together while stirring, then dispense into stock bottles, filtering through a double layer of gauze.

Yield: 500.0 ml.

Gram's Iodine:

NOTE: eye protection must be worn.

Combine 1.7 gm. of Iodine with 3.3 gm. Potassium Iodide and grind fine with a mortar and pestle. Add to 500.0 ml. Deionized water;

small aliquots of the D.I. may be used to dissolve the I/KI mixture and rinse out the mortar.

Then dispense into stock bottles, filtering through gauze.

Yield: 500.0 ml.

Safranin:

Dissolve 1.25 gm. of Safranin in 50.0 ml. of 95% Ethanol.

Add the solution to 450 ml. of Deionized water

Then dispense into stock bottles, filtering through gauze.

Yield: 500.0 ml.

The stains are stored in glass bottles with closures, away from light, at room temperature.

Mark the stock bottles as to their contents, lot # of the stain, date stain was received, date stain was made, date transferred into a secondary vessel (if applicable), and storage conditions. The CDC guidelines and Standard Methods do not define an expiration date; as a point of practice expiration dates will be one year but ,may be extended based on stain clarity and conformity to standards. Once stain is transferred into Koplans jars it expires in 30 days. Complete all elements of the Reagent Log when preparing, entering into use and expiring reagents.

Citations:

<https://www.cdc.gov/meningitis/lab-manual/annex-prep-media-reagents.pdf>