

# A Crystal Violet-Eosin Combination Stain for General Histology

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This is essentially a modification of Gram's procedure securing both stain and counterstain in the same operation by employing a combination staining solution. The stain consists of two solutions which are never mixed.

## A. Staining solution.<sup>1</sup>

Eosin Y, saturated solution in absolute alcohol . . . . .	4 cc.
Crystal violet, saturated solution in absolute alcohol . . . . .	4 cc.
Xylol . . . . .	14 cc.
Absolute alcohol . . . . .	6 cc.

## B. Mordant.

Iodine, saturated solution in xylol . . . . .	1 part
Xylol . . . . .	35 parts

The staining solution should be made up frequently in small amounts since the evaporation of the alcohol will cause it to precipitate, covering the sections with crystals which are hard to remove. For this reason the stains are given as saturated solutions in alcohol since they may easily be kept on hand in this form if the bottles are sealed to keep out moisture. Isopropyl alcohol was used for most of the work with this stain; however, ethyl alcohol should work equally well if allowance is made for its more rapid action.

The stain is applied from the intermediate xylol-alcohol clearing solution. The slide is flooded with the staining solution which is allowed to remain for one to two minutes until the stain is precipitated due to the evaporation of the alcohol. The completion of this precipitation is indicated by the clearness of the remaining solution and the greenish sheen of the precipitated stain. Evaporation should not be hastened by blowing on the slide since this usually results in uneven precipitation of the stain.

After the staining is completed, the solution remaining should be drained off and the slide flooded with the mordant for five to ten seconds. If the mordant is allowed to act too long, the eosin will be removed from the tissue. Differentiation is accomplished by washing with a mixture of two-thirds xylol and one-third absolute alcohol. This solution is best kept in a tightly stoppered bottle, since if left in a stender, the solution will lose its strength in a few weeks due to the

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<sup>1</sup> The stains used in developing this technique were manufactured by the Coleman and Bell Company. The eosin was certified under No. CE9 and had a dye content of 89%, while the crystal violet, dye content 85%, was certified under No. CC9.

evaporation of the alcohol. Before mounting, the slide should be washed well in xylol.

This staining procedure may prove to be of some value not only because, with proper practice, it yields a sharply differentiated stain, but also due to the fact that there is a saving in time and materials because the slides must pass through fewer solutions than in the case of aqueous or weak alcoholic stains. In addition, since the staining time is uniform and no allowance need be made for the stain removed by the dehydrating and clearing solutions, skill and judgment are necessary only for differentiating the stain. The saving in time should be especially valuable to teachers who may find it necessary to make a number of slides for class work on short notice.