



2-1. Acid Orcein -- Azure-Eosin (Refs 2, 5)

Staining Procedures For Plastic Embedded Tissue

Verified at the Applications Laboratory of the Biomedical Division, Sorvall Microtomes

SOLUTIONS:

Acid Orcein Solution

0.2g Orcein
100.0ml Ethyl alcohol, 70%
0.6ml Hydrochloric acid, concentrated

Solution is ready for staining immediately and improves on standing. It is stable for many months.

Nocht's Azure-Eosin

See procedure 2-4.

STAINING PROCEDURE:

1. Cut tissue at 2 μ m to 10 μ m. Thicker sections give better three-dimensional picture.
2. Heat slides on 60°C to 80°C hot plate for 10 minutes.
3. Cool slides.
4. Stain in acid orcein solution at 60°C for 30-45 minutes.
5. Rinse in running tap water for 15 minutes.

If sections begin to detach, blow dry, the refix on hot plate for 10 minutes. Cool, then proceed. If sections are overstained, decolorize in absolute alcohol or 0.1% hydrochloric acid alcohol, then tap water wash.

6. Rinse in distilled water.
7. Stain in Nocht's azure-eosin procedure (2-4) for 1 hour.

Blow dry.

Mount.

RESULTS:

Nuclei	deep blue
cytoplasm of epidermis, smooth muscle, and other cells	light blue
collagen	rose pink
elastic fibers	dark brown
mast cell granules and many mucoid substances	purple
eosinophilic granules and erythrocytes	bright red

Warning: Some of the chemicals used for the staining procedures given in this section may be hazardous if misused. For this reason, read and observe all warnings and cautions provided by the manufacturer for each chemical before proceeding with a staining procedure.

Note: In order to prevent sections from loosening from the slides during staining, all sections should be heat-fixed (60°C to 100°C) to the slides for a minimum of 2-5 minutes prior to staining, preferably at the time the sections are mounted on the slides.