2-8. Methyl Ethyl Green -- Pyronin for Plasma Cells (Ref 4)

Staining Procedures For Plastic Embedded Tissue
Verified at the Applications Laboratory of the Biomedical Division, Sorvall Microtomes

SOLUTIONS:

Purification of Methyl Green

Add 2g methyl green (Commission Certified only) to 100ml hot distilled water. When cool, in separatory funnel, extract the solution with 50ml aliquots of chloroform until the chloroform is nearly colorless. When extraction is complete, leave aqueous solution unstoppered overnight to allow for complete chloroform evaporation.

2% Pyronin Y

2.0g Pyronin Y
100.0ml Distilled water

0.1 M Acetic Acid

5.72ml Glacial acetic acid
qs to Distilled water
1000.0ml

0.1 M Sodium Acetate

8.2g Sodium acetate
qs to Distilled water
1000.0ml

Staining Solution pH 4.8

2.0ml Methyl green, 2%
3.0ml Pyronin Y, 2%
1.0ml Ethanol, absolute
26.4ml Sodium acetate, 0.1 M
17.6ml Acetic acid, 0.1 M

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STAINING PROCEDURE:

1. Rinse in distilled water.
2. Stain one slide at a time in methyl green -- pyronin stain for 5 minutes.
3. Rinse in two changes of distilled water using 1-2 dips.
4. Dehydrate in 95% ethanol for 30 seconds.

Blow dry.
Mount.

RESULTS:

Nuclei, but not most nucleoli, stain with methyl green. Most nucleoli, cytoplasm of plasma cells, plasmacytes, plasmablasts and large pyroninophilic cells are basophilic stem cells.

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 (60ºC to 100ºC) to the slides for a minimum of 2-5 minutes prior to staining, preferably at the time the sections are mounted on the slides.

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