

F-375 MOLLIER'S QUADRUPLE STAIN

Fixation: Any suitable fixative for "Azan" stains.

Sections: Paraffin

Staining:

1. Transfer sections from 70% alcohol to **Orcein, 1%, in Acid Alcohol** (F-375-1) and stain for 12 hours. Wash in distilled water until free color is removed.
2. Stain in Weigert's working Iron Hematoxylin for 12-30 minutes.

*To prepare Weigerts' Hematoxylin solution

*Prepare fresh just before use.

Mix equal parts of **Weigert's Iron Hematoxylin A** (F-375--5), with
Weigert's Iron Hematoxylin B (F-375-4).

3. Rinse in distilled water.
4. Differentiate briefly in **Acid Alcohol, 1%** (F-375-9). Wash 6 minutes in running water.
5. Stain in **Azocarmine G Solution, 0.1%** (F-375-2) for 15-30 minutes. Check under microscope and do not over stain. Rinse in distilled water.
6. Place sections in **Phosphotungstic Acid, 5%** (F-375-10) for 3-6 hours, changing 2 or 3 times, until collagen fibers are completely decolorized. Rinse in distilled water.
7. Stain in **Naphthol Green B, 1%** (F-375-3) for 15-30 minutes.
8. Transfer directly to 95% alcohol and keep sections in motion for 30 seconds to avoid precipitation of Naphthol Green B.
9. Dehydrate, clear and mount in Balsam.

Results:

Elastic fibers	Clear cut black
Collagen fibers	Green
Skeletal muscle	Bright red
Smooth muscle	Pale red
Epithelium	Red
Chromatin of nuclei	Black

References:

Mollier, G., Z. Wissen, Mikr. 55:472, 1938

Clark, G. (ed) Staining Procedures, 3rd ed. Williams & Wilkins, c1973, p 64

