

G-451, MALLORY-HEIDENHAIN AZAN AFTER PERMANGANATE OXIDATION

Fixation: Any, with the exception of alcoholic fixatives.

Sections: Paraffin at 3-5 microns.

Staining:

1. Deparaffinize and hydrate to distilled water.
2. a) For fixatives containing Picric Acid – Wash in running water to remove all traces of Picric Acid.
b) For fixatives containing Mercuric Chloride – Place in Lugol's Iodine (G-451-9) for 15 minutes, rinse in distilled water and clear in **Sodium Thiosulfate, 5%** (G-451-10) for 13 minutes. Wash for 10 minutes or longer in water.
3. Oxidize sections in acidified Permanganate Solution* for 30-60 seconds.
*Prepared by adding 0.5 ml of conc. Sulfuric Acid (G-451-6) to 100 ml of **Potassium Permanganate, 0.25%** (G-451-5) just before use.
4. Bleach in either **Potassium Metabisulfite, 5%** (G-451-7) or Oxalic Acid, 5% (G-451-8) for 1 minute or until sections are colorless. Wash 5 minutes in running water.
5. Stain in **Azocarmine G Solution** (G-451-1) for 45-60 minutes at 56° in a covered staining dish.
6. Wash in running water for approximately 1 minute to differentiate. If section is too dark, complete differentiation in Aniline Alcohol (G-451-2) until acinar parenchyma becomes nearly completely decolorized and the beta cells stand out red against the pinkish alpha cells, rinse in distilled water.
7. Mordant for 5 minutes or more in Ferric **Ammonium Sulfate 5%** (G-451-3). Rinse briefly in distilled water.
8. Stain in **Aniline Blue-Orange G Solution** (G-451-4) for 2-20 minutes under microscopic control. Rinse briefly and blot.
9. Differentiate and dehydrate in absolute alcohol. To intensify orange colors which may be extracted during this step, dehydrate in **Orange G Solution** (G-451-11) and use second bath of absolute alcohol to wash out unbound dye.
10. Clear and mount.

Results:

Alpha cells	Coarse, bright red granules
Beta Cells	Cannot be distinguished
D-cells	Cannot be distinguished

References:

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Foot, N.C., & Menard, M.C., Arch. Path. & Lab Med. 4:211-4, 1927

Mallory, F.B. Pathological Technique, W.B. Saunders Co., Philadelphia, 1938

Clark, G. (ed), Staining Procedures, 3rd edit., The Williams & Wilkins Co., Baltimore, c 1973, p.67.