

O-921, PERMANGANATE/AB/PAS/OG STAIN

FIXATION: After bisecting the gland and then bisecting each half, fix in Formalin-Calcium Acetate (F-117) for 6 - 10 hours followed by 4- 6 hours in 70% alcohol (F-13) before beginning dehydration and embedding.

SECTIONS: Paraffin at 3 - 5 microns.

STAINING:

1. Deparaffinize and hydrate to distilled water.
2. Oxidize for 1 minute in **Acidic Potassium Permanganate Solution** (0-921-1).
3. Place in **Potassium Metabisulfite 1%** (0-921-2) for 1 minute. Wash in running water for 5 minutes.
4. Stain in **Alcian Blue Solution** (0-921 -3) for 10 - 15 minutes. Rinse briefly and vigorously in water followed by a separate wash in running water for 5 minutes.
5. Oxidize for 10 minutes in **Periodic Acid 1%** (O-921-6). Wash for 5 minutes in running water.
6. Stain for 10 minutes in **Schiff's Reagent** (0-921-4). Wash for 5 minutes in running water.
7. Stain for 5 seconds in **Phosphomolybdic Acid-Orange G Solution** (0-921-5). Rinse in water to remove excess Orange G.
8. Dehydrate and then clear in xylene. Examine the sections while in xylene to determine whether the Orange G staining is too weak or too intense, and then, if necessary, rehydrate and either restain in **Orange G Solution** (0-921-5) or rinse again in water.
9. Mount with a synthetic mounting medium.

STAINING RESULTS:

ACTH/MSH cells	Red (many have a blue-purple coloration if step 4 is not done properly or if pH is not low enough.)
TSC cells turquoise.	Turquoise to chromophobic; large lipofuchsin are colored blue-purple to turquoise.
Gonadotropin cells	Turquoise, intense navy blue
GH cells	Dull orange (pink with insufficient Orange G)

REFERENCES:

Herlant, M. and Pasteels, J.L., Methods and Achievements in Experimental Pathology, 3: 25k0-305, 1967

Clark, G., (ed.), Staining Procedures, 3rd edition, Williams & Wilkins, Baltimore, p. 169, c 1973