
K-697, HOLMES' METHOD FOR NERVE CELLS AND FIBERS

Fixation: 10% Buffered Neutral Formalin (F-113)

Sections: Paraffin @ 6 microns.

Staining:

1. Deparaffinize and hydrate to distilled water.
2. Treat with **Silver Nitrate, 20%** (K-697-1) for 2 hours. Rinse in 3 changes of distilled water for 10 minutes.
3. Place in **Impregnating Solution** (K-697-2) overnight at 37°C in a covered vessel.
4. Remove slides, drain excess solution and place in reducing solution for at least 2 minutes.

To prepare reducing solution mix:

<u>Hydroquinone</u> (K-697-3)	1.0 gm
<u>Sodium Sulfite</u> (K-697-4)	10.0 gm
Distilled water	100 ml

Prepare just before use - reducing solution will only keep a few days.

5. Wash in running water for 3 minutes, then rinse in distilled water.
6. Tone in **Gold Chloride, 0.2%** (K-697-5) for 3 minutes. Rinse briefly in distilled water.
7. Develop in **Oxalic Acid, 2%** (K-697-6) under the microscope until axons are thoroughly blue-black. Rinse in distilled water.
8. Place in **Sodium Thiosulfate, 5%** (K-697-7) for 5 minutes, then wash in running water for 5 minutes.
9. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, two changes.
10. Mount with **Permount** (M-18).

Staining Results:

Axis cylinders	Blue to black
Nerves and nerve endings	Black
Background	Gray to rose

References: Luna, L.G. (ed.) AFIP Manual of Histologic Staining Methods, 3rd ed., McGraw-Hill, NY, c 1968, p. 196. Holmes W., Anat. Rec., 86: 157-187, 1943.

