

K-691, BIELSCHOWSKY'S METHOD

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

Sections: Paraffin @ 6 microns

Staining:

1. Deparaffinize and hydrate to distilled water.
2. Place in **Silver Nitrate, 2%** (K-691-1) for 48 hours, in the dark. Rinse quickly in double distilled water.
3. Ammoniacal Silver Solution for 10-20 minutes or until sections turn a deep brown. Rinse in distilled water.

Prepare the Ammoniacal Silver Solution in the following manner
(use chemically clean glassware!)

Add 15 drops of **Sodium Hydroxide, 40%** (K-691-3) to 15 ml of
Silver Nitrate, 10% (K-691-2) Dissolve the resulting precipitate by adding
Ammonium Hydroxide conc. (K-691-4) dropwise until the precipitate
is just dissolved. Store solution mechanically or shake flask frequently
during addition of the Ammonium Hydroxide. Filter and dilute to 60 ml
with distilled water. Make fresh, just before use.

4. Reduce in **Formalin, 20%** (K-691-6) for 5 minutes. Sections appear a dark brownish black. Rinse thoroughly in distilled water.
5. Tone in **Gold Chloride Solution** (K-691-5) for one hour. Sections will be a reddish violet color. Rinse in distilled water.
6. **Sodium Thiosulfate, 5%** (K-691-7) for 1 minute. Wash in water.
7. Dehydrate in 95% Alcohol, absolute alcohol and clear in xylene, two changes each.
8. Mount with **Permount** (M-18)

Results:

Intracellular neurofibrils	Black
Axis cylinders	Black
Dendrites	Black
Background	Purplish

- NOTE: The nuclei of cells other than neurons are impregnated to variable degrees. Therefore, caution must be exercised in the interpretation of the appearances that may be found in tumors and other pathological conditions. In cases of marked gliosis, impregnation of glial fibers may occur.

References:

Luna, L. ed.

AFIP Manual of Histological Staining Methods, 3rd ed.

New York: McGraw Hill Publications, c. 1968, p. 193.

Mallory, F.B. , Pathological Technique, Hafner Publishing Co., New York, 1961, pp. 158-160.