

K-659, SEVIER-MUNGER METHOD FOR NEURAL TISSUES

Fixation: 10% Buffered Neutral Formalin (F-113). Avoid chromate fixatives.

Sections: Paraffin @ 6 microns

PREPARATION OF AMMONICAL SILVER SOLUTION:

Add **Ammonium Hydroxide** (K-659-1B) dropwise to 50ml of 10% **Silver Nitrate** (K-659-1A) until the forming dark brown precipitate has almost disappeared. (Avoid adding an excess of Ammonium hydroxide-shake or stir vigorously during the addition.) Add 0.5ml of **Sodium Carbonate Solution** (K-659-1C), followed by 25 drops of **Ammonium Hydroxide** (K-659-1B), with vigorous shaking or stirring during both additions. Solution should now be clear. Filter and use at once or store in the refrigerator in a well sealed amber bottle. Prepare fresh. Use chemically clean glassware.

STAINING:

1. Deparaffinize and hydrate to distilled water.
2. Place slides in **Silver Nitrate, 20%** (K-659-2) preheated to 60°C and leave in for 15 minutes at 60°C. Rinse one slide at a time in distilled water, then place in a clean coplin jar.
3. Add 10 drops of **Formalin, 2%** (K-659-3) to the Ammoniacal Silver Solution, shaking gently during the addition. Pour this solution over the slides and let develop until golden brown, 5-30 minutes. Keep in motion to avoid precipitate. Examine microscopically - do not wash. Developing should be neither too fast or too slow.
4. Rinse well in 3 changes of water.
5. Place for 2 minutes in **5% Sodium Thiosulfate** (K-659-4). Wash in water.
6. Dehydrate in 95% alcohol, absolute alcohol, and clean in xylene, two changes each.
7. Mount with **Permount** (M-18).

Stain Results:

Large and small peripheral neurites	Black
Axons	Black
Argentaffin granule	Black
Myelin sheath	Light brown
Collagen and muscle	Brown

References:

Sevier, A.C. and Munger, B.L., *J. Neuropath. Exp. Nuerol.*, 24:130-135., 1965.

Luna, L.G., (ed), *Manual of Histologic Staining Methods of the AFIP*, 3rd edition, McGraw-Hill, NY, p. 215., c 1968.