

# Wilder Modification of the Bielschowsky Stain for Reticulum

**Catalog #:** 26392-Series

### Fixation:

10% Buffered Neutral Formalin (#15740) or Russell's Zenker (#64123-05).

### Sections:

Cut paraffin at 6µm sections.

### Staining Procedures:

1. Deparaffinize and hydrate slides to distilled water.
2. Oxidize in Phosphomolybdic Acid, 10% (#26392-07) for 1 minute. Rinse well in distilled water or sunning water to prevent the cells from holding yellow colors.
3. Sensitize in Uranyl Nitrate, 1% (#26392-08) for 1 minute or less. Rinse 10 – 20 seconds in distilled water.
4. Stain in fresh Ammonical Silver for one minute (changing solution frequently). The solution may be prepared as follows:
  1. Add concentrate Ammonium Hydroxide (#26392-02) to 5.0 ml of Silver Nitrate, 10.2% (#26392-01) drop by drop, until the forming precipitate is almost dissolved.
  2. Add 5.0 ml of Sodium Hydroxide, 3.1% (#26392-03) and just dissolve the resultant precipitate by slowly adding conc. Ammonium Hydroxide dropwise. Bring volume up to 50 ml with distilled water.
  3. Use at once or filter and store in refrigerator in amber bottle.
5. Dip quickly in 95% alcohol and transfer to freshly prepared reducing solution. Prepare the reducing solution as follows:
  1. Mix 50.0 ml distilled water, 1.5ml Uranyl Nitrate, 1% (#26392-08) and 0.5 ml of Formalin, 40% neutralized (#26392-04).
  2. Prepare just before use and change frequently during the process.
6. Rinse in distilled water.
7. Tone in Gold Chloride, 0.2% (#26392-09) approx. 1 minute, until the yellow brown color leaves the sections and they turn lavender. Check the slides at this point individually with a microscope to prevent over toning; this could lead to red sections.
8. Rinse in distilled water and transfer to Sodium Thiosulfate, 5% (#26392-10) – approx. 1 minute – to fix the color. Wash in tap water.
9. Counter-stain in Nuclear Fast Red (#26392-06) if desired, rinsing well in distilled water. Alternatively Harris Hematoxylin (#26392-05) with either Van Gieson (#26392-5A) or phloxine can be used as a counterstain.
10. Dehydrate in 95% and 100% alcohol, 2 changes and clear in xylene, 2 changes.
11. Mount. If celloidin is used, clear in oil of origanium followed by 25% alcohol, usually mounted in balsam with Permount.

### Stain Results:

Reticulum fiber (If beading is observed and/or dark background, replace the ammonium Hydroxide Silver Nitrate solution and the reducing solution	Black
Collagen	Rose
Other tissue elements	Red

## **References:**

Wilder, H.C., Amer. J. Clin. Path., 11:817, 1935.

Mallory, F.B. Pathological Technique, Phil., W.B. Saunders & Co, c. 1938

AFIP Manual of Histological Staining Techniques, NY, McGraw-Hill Publ., c. 1968/3rd ed. P.92