

## **F-392, WILDER MODIFICATION OF THE BIELSCHOWSKY STAIN FOR RETICULUM**

**FIXATION:** 10% Buffered Neutral Formalin, Zenker's or Helly's

**SECTIONS:** Paraffin, 6 microns

**STAINING:**

1. Deparaffinize and hydrate to distilled water.
2. Oxidize in **Phosphomolybdic Acid, 10%** (F-392-5) 1 minute. Rinse well in D-H<sub>2</sub>O or running water to prevent the cells from holding a yellow color.
3. Sensitize in **Uranyl Nitrate, 1%**, (F-392-7) for 1 minute or less. Rinse 10-20 seconds in D-H<sub>2</sub>O.
4. Stain in the fresh Ammonical Silver for one minute (changing solution frequently.) The solution may be prepared as follows:
  - a. Add conc. **Ammonium Hydroxide** (F-392-1B) to 5.0 ml of **Silver Nitrate, 10.2%** (F-392-1A) drop by drop, until the forming ppt. is almost dissolved.
  - b. Add 5.0 ml of **Sodium Hydroxide, 3.1%** (F-392-1C) and just dissolve the resultant ppt. by slowly adding conc. Ammonium Hydroxide, dropwise. Bring volume up to 50 ml with distilled water.
  - c. 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:
  - a. Mix 50.0 ml distilled water, 1.5 ml **Uranyl Nitrate, 1%**, (F-392-7) and 0.5 ml of **Formalin, 40%, neutralized** (F-392-2).
  - b. Prepare just before use and change frequently during the process.
6. Rinse in distilled water.
7. Tone in **Gold Chloride, 0.2%** (F-392-9) approx 1 min, 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%** (F-392-10) (approx 1 minute) to fix the color. Wash in tap water.
9. Counterstain in **Nuclear Fast Red**, (F-392-4) if desired, rinsing well in distilled water. Alternatively **Harris Hematoxylin** (F-392-3A) with either Van Geison's (F-392-3B) 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** (M-18)

**Stain Results:**

Reticulum fibers (If beading is observed and/or a 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 Techniques, Phil., W.B. Saunders & Co. c1938

AFIP Manual of Histological Staining Techniques, NY, McGraw-Hill Publ.

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Clark, D., (ed.) Staining Procedures, 3<sup>rd</sup> ed. Baltimore, Williams & Wilkins, c. 1973, p.70

