

F-394, MANUEL'S METHOD FOR RETICULUM

FIXATION: **Buffered Neutral Formlin, 10%** (F-113)

SECTIONS: Paraffin @ 6 microns

STAINING:

1. Deparaffinize and hydrate to distilled water.
2. Sensitize in **Zinc Formalin w/ Zinc sulfate (F-394-7)** for 2 minutes. Dip quickly in running water.
3. Impregnate in **Ammoniacal Silver Solution*** for 1 minute. Wash in running water until no more white precipitate appears in the water.

* To prepare the **Ammoniacal Silver Solution:**

- A. To 93ml **Silver Nitrate, 10%** (F-394-1A) add the **Ammonium Hydroxide, conc.** (F-394-1B) drop by drop (approximately 6ml) while shaking the flask vigorously until the solution clears (solution becomes dark then clears.)
 - B. **Slowly** add 5ml of **Silver Nitrate, 10%** (F-394-1A) add the **Ammonium Hydroxide Silver Nitrate** mixture. The solution should become slightly cloudy, if not, add up to an additional 2 ml of the **Silver Nitrate, 10%** (F-394-1A).
 - C. Let the solution stand overnight and filter before using. Store the solution in the refrigerator.
4. Develop in **Formlin Solution, 1%** (F-394-5) for 1 minute. Wash in running water.
 5. Tone in **Gold Chloride, 1%** (F-394-2) for 1 minute. Wash in running water.
 6. Reduce in **Sodium Thiosulfate, 5%**(F-394-6) for 1 minute. Wash in running water.
 7. Counterstain with **Nuclear Fast Red** (F-394-3) for 5 minutes. Rinse in three changes of distilled water.
 8. Dehydrate in 95% alcohol, absolute alcohol, and clear in **Xylene** (C-120) two changes each.
 9. Mount.

STAINING RESULTS:

Reticulum	Black
Nuclei	Red
Background	Red

REFERENCES:

Ed. L. Luna, Manual of Histology Staining Methods of the AFIP, 3rd. ed. McGraw-Hill Pub., NY. P89.c1968