



## WILDER'S RETICULUM STAIN KIT PROCEDURE

Item# **CUKTWRE**

(Revised 03/7/18)

**KIT COMPONENTS INCLUDED:**

100ml 1% POTASSIUM PERMANGANATE  
100ml 1% URANYL NITRATE  
100ml 1% GOLD CHLORIDE  
100ml 3.1% SODIUM HYDROXIDE

100ml 25% HYDROBROMIC ACID  
100ml 10.2% SILVER NITRATE  
100ml 5% SODIUM THIOSULFATE

**PRINCIPLE:** This kit demonstrates reticulum fibers.

**SPECIMEN:** Any well fixed paraffin embedded tissue cut at 4 to 5 microns; use strong tissue adhesive or charged slides!

**QUALITY CONTROL:** American MasterTech Scientific Recommended Control Slide: Reticulum Fibers, CSR0125P.

**SOLUTIONS:**

**AMMONIACAL SILVER SOLUTION: Prepare at Step 6.**

Into chemically cleaned glassware or unused plasticware add 5.0ml of 10.2% SILVER NITRATE. Next add Concentrated Ammonium Hydroxide (*Not in Kit*) drop by drop, mixing solution thoroughly between drops, until the precipitate is substantially dissolved. Slowly add 5 ml of 3.1% SODIUM HYDROXIDE and mix thoroughly. Then add enough Concentrated Ammonium Hydroxide, drop by drop, to just dissolve the remaining precipitate. Add distilled water to obtain a total volume of 50 ml and mix thoroughly. Solution may be filtered if desired.

**REDUCING SOLUTION: Prepare at Step 9**

DISTILLED WATER..... 50 ml.  
37% FORMALDEHYDE (*Not supplied*) 0.5 ml.  
1% URANYL NITRATE ..... 1.5 ml.  
Add 37% Formaldehyde to Distilled Water and mix.  
Add 1% URANYL NITRATE and mix thoroughly.  
*Reducing Solution* may be filtered if desired.

**PROCEDURE:**

1. Deparaffinize slide using Xylene or a Xylene Substitute and hydrate through alcohols.
2. Rinse slide thoroughly in Tap water, followed by 2 changes of Distilled water.
3. Place slide in 1% POTASSIUM PERMANGANATE for 5 minutes.
4. Rinse slide in Distilled water.
5. Place slide in 25% HYDROBROMIC ACID for 1 minute.
6. Rinse slide in running Tap water, followed by 1 change of Distilled water.
7. Place slide in 1% URANYL NITRATE for 1 minute; **do not re-use this solution**.
8. Rinse slide in Distilled water for 15 seconds.
9. Place slide in *Ammoniacal Silver Solution* for 1 minute.
10. Rinse slide quickly in 95% Reagent alcohol.
11. Place slide in *Reducing Solution* and agitate gently for 1 minute. Reduce no more than 2 slides at a time. Replace *Reducing Solution* after each use.
12. Rinse slide gently in 2 changes of Distilled water.
13. Place slide in 1% GOLD CHLORIDE for 1 minute or until section becomes gray-lavender. Control toning by rinsing slide in Distilled water and check microscopically for black reticulum fibers against a gray-lavender background.
14. Rinse slide gently in 2 changes of Distilled water.
15. Place slide in 5% SODIUM THIOSULFATE for 1 to 2 minutes.
16. Rinse slide gently in Tap water for 5 minutes.
17. Place slide in *Nuclear Fast Red Stain (Not supplied)* for 1 to 5 minutes.
18. Rinse slide in Tap water.
19. Dehydrate through 3 changes of fresh Absolute Alcohol.
20. Clear through 3 changes of fresh Xylene or Xylene Substitute.
21. Coverslip slide using a permanent mounting media.

**RESULTS:**

Reticulum Fibers: **GRAY TO BLACK**  
Nuclei: **RED**  
Background: **PINK**

**REFERENCE:** Sheehan DC Hrapchak BB: Theory and Practice of Histotechnology; 1980, 182-183.

Copyright ©2012-2018 American MasterTech Scientific. All rights reserved.

No part of these pages may be used for any purpose other than personal use. Therefore, reproduction, modification, storage in a retrieval system or retransmission, in any form or by any means, for reasons other than personal use, is strictly prohibited without prior written permission.