

## INTENDED USE

Reticulum Stain is intended to demonstrate reticular fibers. Reticulum Stain reagents are for "In Vitro Diagnostic Use."

The main function of reticular fibers is to provide support. They are normally found throughout the body, particularly in liver, lymph node, spleen and kidney.<sup>1</sup> Ammoniacal silver stains are the most commonly used methods for demonstration of reticular fibers. In the procedure of Gordon and Sweets, tissue sections are oxidized by potassium permanganate with oxalic acid removing the excess potassium permanganate. Ferric ammonium sulfate acts as the sensitizer. After the silver impregnation, formalin is used to reduce the silver to its visible metallic form. Gold chloride tones the sections and any unreduced silver is removed by sodium thiosulfate. A counterstain may be used, if desired.<sup>2</sup>

## REAGENTS

**SODIUM HYDROXIDE SOLUTION**, Catalog No. HT1021-100 ml  
3% aqueous solution

**POTASSIUM PERMANGANATE SOLUTION**, Catalog No. HT1022-100 ml  
1% aqueous solution

**OXALIC ACID SOLUTION**, Catalog No. HT1023-100 ml  
1% aqueous solution

**FERRIC AMMONIUM SULFATE SOLUTION**, Catalog No. HT1024-500 ml  
2.5% aqueous solution

**SILVER NITRATE SOLUTION**, Catalog No. HT1025-50 ml  
10% aqueous solution

**GOLD CHLORIDE SOLUTION**, Catalog No. HT1026-100 ml  
0.2% aqueous solution

**SODIUM THIOSULFATE SOLUTION**, Catalog No. HT1027-500 ml  
5% aqueous solution

### STORAGE AND STABILITY:

Store unopened Reticulum Stain kit in refrigerator (2–8°C). After opening, Sodium Hydroxide, Potassium Permanganate, Oxalic Acid, Ferric Ammonium Sulfate and Sodium Thiosulfate Solutions may be stored in the refrigerator or at room temperature (2–26°C). Store Silver Nitrate and Gold Chloride Solutions in refrigerator (2–8°C). Reagents are stable until the expiration dates shown on the labels.

Silver Nitrate Working Solution should be used once and then discarded.

Nuclear Fast Red solution, Catalog No. N3020, nuclear fast red 0.1% in 5% aluminum sulfate, should have limited exposure to air. Material oxidizes when exposed to air. Repeat exposure can cause the solution to become more alkaline over time, causing aluminum sulfate to fall out of solution.

In dry form ammoniacal salts may present an explosive hazard. Do not allow ammoniacal silver solution to dry out. Store used ammoniacal silver solution in plastic bottles – do not store in glass. Wallington recommended the inactivation of the ammoniacal silver solution by the addition of dilute hydrochloric acid or sodium chloride solution.<sup>3</sup>

### DETERIORATION:

Potassium Permanganate Solution should be purple. Solution may be used several times, but should be discarded if it turns brown.

### PREPARATION:

To prepare AMMONIACAL SILVER NITRATE SOLUTION:

1. Pipet 5 ml Silver Nitrate Solution in an Erlenmeyer flask.
2. In a hood, while shaking or swirling the flask continuously, add concentrated ammonium hydroxide, drop by drop, until the precipitate it formed is completely dissolved. Do not add excess ammonium hydroxide.
3. Add 5 ml Sodium Hydroxide Solution to the flask. Solution will turn black and precipitate will form. Continuously swirl the flask and add concentrated ammonium hydroxide, drop by drop, until the precipitate just dissolves. At this stage the solution should not be completely clear.

NOTE: If no cloudiness remains, add Silver Nitrate Solution drop by drop, until one drop causes the solution to become permanently cloudy. Only a faint cloudiness is desirable.

4. Dilute the resulting solution to 50 ml with distilled or deionized water. Filter into a chemically clean Coplin jar. Use once and then discard.

10% Formalin Solution is prepared by diluting 5 ml formaldehyde, 37–40%, with 45 ml distilled or deionized water. Prepare fresh daily.

The Potassium Permanganate Solution and the Oxalic Acid Solution should be divided into two 50-ml aliquots. Each aliquot can be used 5 times and then discarded. Do not mix used reagent with unused solution in original bottle.

Other reagents are supplied ready for use.

### PRECAUTIONS:

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet and product labeling for any updated risk, hazard or safety information.

Reticulum TISSUE-TROL™ control slides are paraffin embedded human tissue containing reticulum and should be considered potentially infectious.

## PROCEDURE

### SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No known test method can offer complete assurance that blood

samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Fix specimen in 10% neutral buffered formalin, process and embed in paraffin. Cut paraffin sections at 4 to 5 microns. Incorporate appropriate controls.

### SPECIAL MATERIALS REQUIRED, BUT NOT PROVIDED:

Positive control slides, such as Sigma Reticulum TISSUE-TROL™ slides, Catalog Nos. R4768 or TTR010, should be included in each run

Counterstain (optional) Eosin Y solution or Nuclear Fast Red Solution, Catalog No. N3020, nuclear fast red 0.1% in 5% aluminum sulfate

Ammonium Hydroxide Solution, concentrated

Ethanol, absolute

Formalin Solution, 10%

Reagent Alcohol

Xylene or xylene substitute

Forceps, plastic or paraffin coated

Coplin jars, chemically clean

### NOTE:

The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

### PROCEDURE:

1. Deparaffinize sections and hydrate to distilled water.
2. Oxidize sections in Potassium Permanganate Solution for **5 minutes**.
3. Rinse slides in tap water for **2 minutes**.
4. Bleach in Oxalic Acid Solution for **2 minutes** or until sections are colorless
5. Wash slides in tap water for **2 minutes**.
6. Sensitize sections in Ferric Ammonium Sulfate Solution for **15 minutes**.
7. Wash slides in several changes of distilled water.
8. Impregnate sections with Ammoniacal Silver Nitrate Solution for **2 minutes**.
9. Rinse slides well with distilled water.
10. Reduce sections for **2 minutes** in 10% Formalin Solution.
11. Wash slides in tap water for **3 minutes**.
12. Tone sections in Gold Chloride Solution for **10 minutes**.
13. Rinse slides in distilled water.
14. Place slides in Sodium Thiosulfate Solution for **1 minute**.
15. Wash slides in tap water for **2 minutes**.
16. Counterstain, if desired, with Nuclear Fast Red Solution for **3–5 minutes** or an Eosin Y Solution for **1–2 minutes**. Generally, all sections except those from liver are counterstained. Wash well in water.
17. Dehydrate in two changes each of 95% ethanol and absolute ethanol.
18. Clear in xylene and mount with synthetic resin.

## PERFORMANCE CHARACTERISTICS

Reticulum – Black

Background – Pink to rose (if counterstained with Nuclear Fast Red)

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

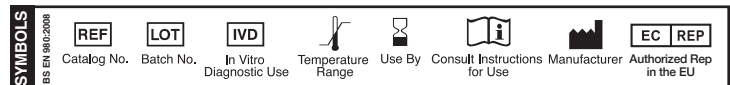
## REFERENCES

1. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd ed, CV Mosby Co., St. Louis, MO, 1980, pp 181–182
2. Carson FL: Histotechnology: A Self Instructional Text, ASCP Press, Chicago, IL, 1990, pp 150–155
3. Wallington, EF (1965): The explosive properties of ammoniacal-silver solutions. J Med Lab Technol, 22, 220–223

TISSUE-TROL is a trademark of Sigma-Aldrich Co. LLC

2014 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at [www.sigmaaldrich.com](http://www.sigmaaldrich.com) and/or on the reverse side of the invoice or packing slip.

Procedure No. HT102  
Previous Revision: 2016-11  
Revised: 2020-02



**EC REP** MDSS GmbH  
Schiffgraben 41  
30175 Hannover, Germany



SIGMA-ALDRICH, INC.  
3050 Spruce Street, St. Louis, MO 63103 USA  
314-771-5765

Technical Service: 800-325-0250  
or e-mail at [clintech@sial.com](mailto:clintech@sial.com)  
To Order: 800-325-3010  
[www.sigma-aldrich.com](http://www.sigma-aldrich.com)