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## INTENDED USE

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Sigma-Aldrich 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>

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## REAGENTS

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**SODIUM HYDROXIDE SOLUTION**, Catalog No. HT102-1  
3% aqueous solution (100 ml)

**POTASSIUM PERMANGANATE SOLUTION**, Catalog No. HT102-2  
1% aqueous solution (100 ml)

**OXALIC ACID SOLUTION**, Catalog No. HT102-3  
1% aqueous solution (100 ml)

**FERRIC AMMONIUM SULFATE SOLUTION**, Catalog No. HT102-4  
2.5% aqueous solution (500 ml)

**SILVER NITRATE SOLUTION**, Catalog No. HT102-5  
10% aqueous solution (50 ml)

**GOLD CHLORIDE SOLUTION**, Catalog No. HT102-6  
0.2% aqueous solution (100 ml)

**SODIUM THIOSULFATE SOLUTION**, Catalog No. HT102-7  
5% aqueous solution (500 ml)

### 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.

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, HT102-5, 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, Catalog No. HT102-1, 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, Catalog No. HT102-5, 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 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.

### US Risks and Safety Statements

Sodium Hydroxide Solution is CORROSIVE. Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Take off immediately all contaminated clothing. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Potassium Permanganate Solution. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Caution: Avoid contact and inhalation.

Oxalic Acid Solution is CORROSIVE. Causes burns. Harmful in contact with skin and if swallowed. Possible risk of harm to the unborn child. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Take off immediately all contaminated clothing. Wear suitable protective clothing, gloves and eye/face protection. Target organs: Kidneys and nerves.

Ferric Ammonium Sulfate Solution is an IRRITANT. Irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves.

Silver Nitrate Solution. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Caution: Avoid contact and inhalation. Target organs: Blood and nerves.

Gold Chloride Solution. Caution: Avoid contact and inhalation.

Sodium Thiosulfate Solution is an IRRITANT. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves.

10% Formalin Solution is HARMFUL. Harmful by inhalation, in contact with skin and if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with skin and if swallowed. May cause sensitization by inhalation and skin contact. Do not breathe vapor. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Potential cancer hazard. Contains formaldehyde. Readily absorbed through skin. Target organs: Eyes and kidneys. Calif. Prop. 65 Carcinogen.

Ammonium Hydroxide Solution is CORROSIVE and Dangerous for the Environment. Harmful if swallowed. Causes burns. Very toxic to aquatic organisms. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Avoid release to the environment. Refer to special instructions/safety data sheets.

Reagent Alcohol is FLAMMABLE and an IRRITANT. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Target organs: Nerves and liver.

Xylene is FLAMMABLE and HARMFUL. Possible risk of impaired fertility. May cause harm to the unborn child. Harmful by inhalation and in contact with skin. Irritating to respiratory system and skin. Risk of serious damage to eyes. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

### EU Risks and Safety Statements (Caution: Substances not yet fully tested)

Sodium Hydroxide Solution is CORROSIVE. Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Potassium Permanganate Solution. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Avoid release to the environment. Refer to special instructions/safety data sheets. Avoid contact with skin and eyes. Do not breathe vapor.

Ferric Ammonium Sulfate Solution. Avoid contact with skin and eyes. Do not breathe spray.

Silver Nitrate Solution. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Avoid contact with skin and eyes. Do not breathe vapor.

Gold Chloride Solution. Avoid contact with skin and eyes. Do not breathe spray.

Sodium Thiosulfate Solution. Avoid contact with skin and eyes. Do not breathe spray.

10% Formalin Solution is HARMFUL. Harmful by inhalation, in contact with skin and if swallowed. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact. Wear suitable protective clothing and gloves.

Ammonium Hydroxide Solution is CORROSIVE and Dangerous for the Environment. Causes burns. Very toxic to aquatic organisms. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Avoid release to the environment. Refer to special instructions/safety data sheets.

Reagent Alcohol is HIGHLY FLAMMABLE and an IRRITANT. Highly Flammable. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Xylene is HARMFUL. Flammable. Harmful by inhalation and in contact with skin. Irritating to skin. Avoid contact with eyes.

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## PROCEDURE

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### SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. 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 No. R 4768, should be included in each run  
Counterstain (optional) Eosin Y solution or Nuclear Fast Red Solution, Catalog No. N 3020, nuclear fast red 0.1% in 5% aluminum sulfate  
Ammonium Hydroxide Solution, concentrated  
Ethanol, absolute  
Formalin Solution, 10%  
Reagent Alcohol  
Xylene  
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 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.

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**PERFORMANCE CHARACTERISTICS**

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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.

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**REFERENCES**

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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

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