IVD For In-Vitro Laboratory Use Only CE

Issue No: 0617-01 Rev 5
(07/09/22)

# Product Protocol

| Product Name | Reticulin Stain Kit (Gordon & Sweets 1936) (without Gold Chloride) |
|---|---|

| Product Code | RRSK102-100 |
|---|---|

## Reagents

| | |
|---|---|
| Potassium Permanganate 0.5% solution | 2x 50ml |
| Sulphuric Acid 3% solution | 1x 50ml |
| Oxalic Acid 1% solution | 1x 50ml |
| Ferric Ammonium Sulphate 2.5% solution | 1x 50ml |
| Silver Nitrate 10% solution | 1x 50ml |
| Ammonia 33% solution | 1x 50ml |
| Sodium Hydroxide 3% solution | 1x 50ml |
| Formaldehyde 10% (v/v) solution unbuffered | 1x 50ml |
| Sodium Thiosulphate 5% solution | 1x 50ml |
| Neutral Red 0.1% | 1x 50ml |

## General Information

| | |
|---|---|
| Number of Tests: | 100 (based on bench top staining of batches of 10) |
| Procedure Time: | 50 minutes (approximate) |
| Shelf Life: | 3 Years (from date of manufacture) |
| Storage: | Store silver nitrate 2-8 °C<br>Other reagents 15-25 °C |

## Principle

This kit is used to demonstrate reticulin fibres. The Gordon & Sweet method uses metal impregnation providing excellent contrast so even the finest fibres can be seen. Reticulin fibres have poor affinity for silver solutions but treatment with potassium permanganate produces sensitised sites where silver can be deposited. Post treatment with formaldehyde solution causes deposition of the silver ions as silver metal. Excess silver is removed by treatment with sodium thiosulphate

## Specimen Collection

Tissue should be fixed in formaldehyde for best results. Tissue should then be processed and embedded in paraffin wax. Sections should be cut at about 5 microns

## Working Stock Solutions

Ammoniacal Silver (see Note 1):
Pipette 5ml of 10% silver nitrate solution into a chemically clean flask. Add ammonia solution drop by drop, mixing well between drops, until the precipitate formed just dissolves (this point is reached when a faint opalescence is seen). Add 5ml of 3% Sodium hydroxide solution mix and then add Ammonia solution drop by drop until the precipitate just dissolves but still has a faint opalescence.
if at any stage excess ammonia is present, indicated by the presence of opalescence, then add a few drops of 10% silver nitrate to produce a slight precipitate.
Dilute this solution to 50ml with deionised water and filter before use. Solution should be made fresh before use.

Acidified Potassium Permanganate:
Mix 9.5ml of potassium permanganate solution with 0.5ml of sulphuric acid solution. This purple solution will keep of 3-4 weeks but should be discarded after being used 5 times or if it turns brown

## Protocol

1. Dewax sections, hydrate through alcohols and rinse in tap water
2. Oxidise the sections with acidified potassium permanganate for 5 minutes
3. Wash well in tap water
4. Decolourise with oxalic acid solution for 1 minute
5. Wash sections well in tap water
6. Mordant sections in ferric ammonium sulphate solution for 15 minutes

Digitally signed by Ceaicovschi Tudor
Date: 2025.08.29 18:03:52 EEST
Reason: MoldSign Signature
Location: Moldova
MOLDOVA EUROPEANĂ

# Product Protocol

## Protocol (Contd)

7. Wash well with several changes of distilled water
8. Treat with filtered ammoniacal silver solution for 12-15 seconds
9. Wash well in several changes of distilled water (the section must be transluscent)
10. Reduce in the formaldehyde solution for 1-2 minutes, check microscopically and if under impregnated repeat steps 8-10
11. Wash in distilled water
12. Treat with sodium thiosulphate for 5 minutes
13. Wash well in water
14. If counterstain is required stain with neutral red for 30 seconds
15. Rinse in water
16. Dehydrate through alcohols, clear and mount

## Results

| | |
|---|---|
| Reticulin fibres: | Black |
| Nuclei: | Black (lipofuchsin and melanin are also weakly stained) |
| Other Elements: | Red |

## Notes

1. **Caution** Ammoniacal Silver Solutions can present an explosive hazardif dry or in contact with metals. Do not allow to dryout. Inactivate with concentrated sodium chloride solution before discarding. Do not store, it is recomended that a fresh solution is made before use
2. All glassware used in this procedure must be chemically clean and well washed in distilled water. Avoid the use of metal forceps with silver stains. Use plastic forceps or plastic coated metal forceps to handle slides
3. All biological material should be considered potentially hazardous/infectious and should be handled with care. Ensure all specimens (smears or tissue sections) are clearly labelled with suitable identification before processing. Perform all staining procedures using a staining rack or using small volumes of reagent in small coplin jars (plastic 5 slide mailers use about 20ml reagent and make convenient staining containers).
4. Component bottle Sizes have been supplied which are as close as possible to the number of tests needed. There may be occasion where more product than necessary is supplied, please either disgard or use as required
5. Staining times may be modified to provide varying intensities of staining
6. Other counterstains may also be used in this method for example nuclear fast red which is less likely to be removed by alcohol. Staining times may need to be adjusted for different counterstains

## Stability

If correctly stored the reagents are usuable until the expiry date

## Disposal

Hazardous Reagents Included, observe local waste disposal regulations

**Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or the patient is established**

**Atom Scientific Ltd, 2A, East Tame Business Park,Hyde, Cheshire, SK14 4GX, United Kingdom**
**T: +44 (0) 161 366 5123 E: enquiries@atomscientific.com W: www.atomscientific.com**