

1.00251.0007 **REF****Microscopy****Reticulin silver plating kit acc. to Gordon & Sweets**

for the detection of reticular fibers in histological tissue specimens

**For professional use only****IVD** In Vitro Diagnostic Medical Device**Intended purpose**

This "Reticulin silver plating kit acc. to Gordon & Sweets - for the detection of reticular fibers in histological tissue specimens" is used for human-medical cell diagnosis and serves the purpose of the histological investigation of sample material of human origin. It is a ready-to-use staining kit designed to enhance the visibility of target structures (by fixing, embedding, staining, counterstaining, mounting) in histological specimen materials, for example histological sections of e.g. the kidney, the heart, or the liver in combination with other *in vitro* diagnostica from our portfolio.

The Reticulin silver plating kit is used to identify reticular fibers in histological tissue specimens.

Unstained structures are relatively low in contrast and are extremely difficult to distinguish under the light microscope. The images created using the staining solutions help the authorized and qualified investigator to better define the form and structure in such cases. Further examinations may be necessary to reach a definitive diagnosis.

**Principle**

Reticulin fibers, on which metallic silver is able to precipitate, can be visualized by means of silver salts.

The material is first oxidized with potassium permanganate. Metallic silver is formed from the ammoniacal silver nitrate solution, which precipitates as a brownish deposit on the target structures. Formaldehyde, which is a strong reducing agent, accelerates the process. When toning with gold chloride, the metallic silver is transferred to the more stable gold compound, leading to more intensive results.

Nonspecific silver is bound to sodium thiosulfate.

**Sample material**

The tissue specimens are fixed with neutral buffered formalin

A layer thickness of 4 - 5 µm is recommended for iliac crest biopsies and vertebral bodies, and of 2 - 3 µm for other tissues

**Reagents**

Cat. No. 1.00251.0007

Reticulin silver plating kit acc. to Gordon & Sweets

**Package components:**

The staining kit contains

Reagent 1: Potassium permanganate solution	16 ml
Reagent 2: Sulfuric acid	16 ml
Reagent 3: Oxalic acid	30 ml
Reagent 4: Ammonium iron(III) sulfate solution	30 ml
Reagent 5*: Ammoniacal silver nitrate solution	30 ml
Reagent 6: Formaldehyde solution	30 ml
Reagent 7: Gold chloride solution	30 ml
Reagent 8: Sodium thiosulfate solution	30 ml

**\* Important note:**

The pipette kit supplied with the test set is screwed onto the glass bottle (Reagent 5) before first use. In order to avoid contamination, it should not be removed afterwards (silver nitrate solution is very reactive). The bottle should be stored upright with the other test set components according to specification.

**Also required:**

Cat. No. 1.00121	Nuclear fast red-aluminum sulfate solution 0.1 % for microscopy	500 ml
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**Sample preparation**

The sampling must be performed by qualified personnel.

All samples must be treated using state-of-the-art technology.

All samples must be clearly labeled.

Suitable instruments must be used for taking samples and their preparation.

Follow the manufacturer's instructions for application / use.

When using the corresponding auxiliary reagents, the corresponding instructions for use must be observed.

Deparaffinize and rehydrate sections in the conventional manner.

**Reagent preparation**

The solutions used for staining are ready-to-use, dilution of the solutions is not necessary and merely produces a deterioration of the staining result and their stability.

**Procedure**

Staining with the reagents 1 - 8 is performed on a staining rack made of plexiglass or coated with plastic.

Deparaffinize histological slides in the conventional manner and rehydrate in a descending alcohol series.

The reagents are dripped onto the section one after another so as to cover the section completely.

Rinse with distilled water using a washing bottle.

Do not use metal tweezers and do not allow any other metal objects to come into contact with the slides.

The stated times should be adhered to in order to guarantee an optimal staining result.

Slide with histological specimen		
Distilled water		10 sec
Reagent 1 (Potassium permanganate solution) and Reagent 2 (Sulfuric acid) drip one after the other	4 drops of each	5 min
Distilled water		10 sec
Reagent 3 (Oxalic acid)	8 drops	2 min
Distilled water		10 sec
Reagent 4 (Ammonium iron(III) sulfate solution)	8 drops	2 min
Distilled water		10 sec
Reagent 5 (Silver nitrate solution)	4 drops	2 min
Distilled water		10 sec
Reagent 6 (Formaldehyde solution)	8 drops	2 min
Distilled water		10 sec
Reagent 7 (Gold chloride solution)	8 drops	2 min
Distilled water		10 sec
Reagent 8 (Sodium thiosulfate solution)	8 drops	2 min
Distilled water		10 sec
Ethanol 70 %		1 min
Ethanol 96 %		1 min
Ethanol 100 %		1 min
Ethanol 100 %		1 min
Xylene or Neo-Clear™		5 min
Xylene or Neo-Clear™		5 min
Mount the Neo-Clear™-wet slides with Neo-Mount™ or the xylene-wet slides with Entellan™ new and cover glass.		

After dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear™, histological slides can be covered with non-aqueous mounting agents (e.g. Entellan™ new, Neo-Mount™) and a cover glass and can then be stored.

**Result**

Reticular fibers

black

## Procedure "Nuclear staining with nuclear fast red solution"

subsequent to silver impregnation on the staining rack or in the staining cell

Nuclear fast red-aluminum sulfate solution 0.1 %	3 min
Distilled water	10 sec
Dehydrate, clear, and wet-mount the sections in the conventional manner.	

### Result

Reticular fibers	black
Nuclei	red
Background	red
Collagen	red

### Trouble-shooting

Silver-staining techniques can be difficult and require special care during the procedure.

Microscopic image	Possible cause	Remedy
Poor visualization of reticulin fibers	Gold chloride solution incubated too long	See "Procedure"
Poor visualization of reticulin fibers	Slide insufficiently covered with reagent	Increase the number of drops or use a histomarker
Poor visualization of reticulin fibers	Sections too thin	See "Sample material"
Poor or no visualization of reticulin fibers	Incorrect storage / shelf-life of the kit	Observe storage and shelf-life
Unclean slide / precipitation	Insufficiently rinsed	See "Procedure"
Reticulin fibers stained too intensely	Gold chloride solution not incubated long enough	See "Procedure"
Reticulin fibers stained too intensely	Sections too thick	See "Sample material"

### Technical notes

The microscope used should meet the requirements of a medical diagnostic laboratory.

When using histoprocessor systems or automatic staining systems, please follow the instructions for use supplied by the supplier of the system and software.

### Analytical performance characteristics

"Reticulin silver plating kit acc. to Gordon & Sweets" stains and thereby visualizes biological structures, as described in the "Result" chapter of this IFU. The use of the product is only to be carried out by authorized and qualified persons, this includes, among other things, sample and reagent preparation, sample handling, histoprocessing, decisions regarding suitable controls and more.

The analytical performance of the product is confirmed by testing each production batch. The successful participation in international interlaboratory tests on a regular basis provide an additional and unaffiliated confirmation of analytical specificity.

For the following stains, the analytical performance was confirmed in terms of specificity, sensitivity and repeatability of the product with a rate of 100 %:

	Inter-assay Specificity	Inter-assay Sensitivity	Intra-assay Specificity	Intra-assay Sensitivity
Histological staining				
Reticular fibers	9/9	9/9	7/7	7/7

Analytical performance results

Intra- (performed on the same batch) and inter-assay (performed on different batches) data list the number of correctly stained structures in relation to the number of performed assays.

The results of this Performance Evaluation confirms that the product is suitable for the intended use and performs reliably.

### Diagnostics

Diagnoses are to be made only by authorized and qualified personnel. Valid nomenclatures must be used.

This method can be supplementarily used in human diagnostics.

Further tests must be selected and implemented according to recognized methods.

Suitable controls should be conducted with each application in order to avoid an incorrect result.

### Storage

Store the Reticulin silver plating kit acc. to Gordon & Sweets - for the detection of reticular fibers in histological tissue specimens at +2 °C to +8 °C.

The pipette kit supplied with the test set is screwed onto the glass bottle (Reagent 5) before first use. In order to avoid contamination, it should not be removed afterwards (silver nitrate solution is very reactive). The bottle should be stored upright with the other test set components according to specification.

### Shelf-life

The Reticulin silver plating kit acc. to Gordon & Sweets - for the detection of reticular fibers in histological tissue specimens can be used until the stated expiry date.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at +2 °C to +8 °C.

The bottles must be kept tightly closed at all times.

### Capacity

The package is sufficient for up to 110 applications.

### Additional instructions

#### For professional use only.

In order to avoid errors, the application must be carried out by qualified personnel only.

National guidelines for work safety and quality assurance must be followed. Microscopes equipped according to the standard must be used.

### Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

### Instructions for disposal

The package and any unused control slides must be disposed of in accordance with the current disposal guidelines.

Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines. Information on disposal can be obtained under the Quick Link "Hints for Disposal of Microscopy Products" at [www.microscopy-products.com](http://www.microscopy-products.com). Within the EU the currently applicable REGULATION (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 applies.

### Auxiliary reagents

Cat. No.	1.00121	Nuclear fast red-aluminum sulfate solution 0.1 % for microscopy	500 ml
Cat. No.	1.00579	DPX new non-aqueous mounting medium for microscopy	500 ml
Cat. No.	1.00974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No.	1.04699	Immersion oil for microscopy	100-ml dropping bottle, 100 ml, 500 ml
Cat. No.	1.07961	Entellan™ new rapid mounting medium for microscopy	100 ml, 500 ml, 1 l
Cat. No.	1.08298	Xylene (isomeric mixture) for histology	4 l
Cat. No.	1.09016	Neo-Mount™ anhydrous mounting medium for microscopy	100-ml dropping bottle, 500 ml
Cat. No.	1.09843	Neo-Clear™ (xylene substitute) for microscopy	5 l

### Hazard classification

Cat. No. 1.00251.0007

Please observe the hazard classification printed on the label and the information given in the safety data sheet.

The safety data sheet is available on the website and on request.

CAUTION! Contains CMR substances. Please observe the corresponding safety instructions given in the safety data sheet.

### Main product components

Cat. No. 1.00251.0007

Reagent 1	
KMnO <sub>4</sub>	5 g/l
Reagent 2	
H <sub>2</sub> SO <sub>4</sub>	8 g/l
Reagent 3	
C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>	10 g/l
Reagent 4	
(NH <sub>4</sub> )Fe(SO <sub>4</sub> ) <sub>2</sub> × 12 H <sub>2</sub> O	25 g/l
Reagent 5	
AgNO <sub>3</sub>	10 g/l
NH <sub>3</sub>	16 g/l
NaOH	36 g/l
Reagent 6	
CH <sub>2</sub> O	40 g/l

Reagent 7  
HAuCl<sub>4</sub> 0.8 g/l

Reagent 8  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 0.1 mol/l

### General remark

If during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorised representative and to your national authority.

### Literature

1. Romeis - Mikroskopische Technik, Editors: Maria Mulisch, Ulrich Welsch, 2015, Springer Spektrum, 19. Auflage
2. Welsch Sobotta - Lehrbuch Histologie, Editor: Ulrich Welsch, 2006, ELSEVIER Urban&Fischer, 2. Auflage
3. Histotechnik, Gudrun Lang, 2013 Springer Verlag, 2. Auflage
4. Theory and Practice of Histological Techniques, John D Bancroft, Marilyn Gamble, 2008, Churchill Livingstone ELSEVIER, 6th Edition
5. Laboratory Manual of Histochemistry, Linda L. Vacca, 1985, Raven Press
6. Basiswissen Histologie und Zytologie, Karl Heinz Stein, Hellmut Flenker, 1998, uZv, 2. Auflage
7. Histological & Histochemical Methods: Theory & Practice, J. A. Kiernan, 1990, Pergamon Press, 2nd Edition
8. Histological and Histochemical Methods, Theory and practice, J. A. Kiernan, 2015, Scion Publishing Ltd, 5th Edition



H290 May be corrosive to metals.  
H302 + H332 Harmful if swallowed or if inhaled.  
H315 Causes skin irritation.  
H317 May cause an allergic skin reaction.  
H319 Causes serious eye irritation.  
H341 Suspected of causing genetic defects.  
H350 May cause cancer.  
H360D May damage the unborn child.  
H410 Very toxic to aquatic life with long lasting effects.

P273 Avoid release to the environment.  
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.  
P301 + P312 IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell.  
P302 + P352 IF ON SKIN: Wash with plenty of water.  
P304 + P340 + P312 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell.  
P308 + P313 IF exposed or concerned: Get medical advice/ attention.  
P362: Take off contaminated clothing and wash before reuse.  
P390: Absorb spillage to prevent material damage.  
P391: Collect spillage.  
P405: Store locked up.  
P406: Store in corrosive resistant container with a resistant inner liner.  
P501: Dispose of contents/ container to an approved waste disposal plant.

Reagent 1:  
H411: Toxic to aquatic life with long lasting effects..

Reagent 2:  
H290: May be corrosive to metals.

Reagent 4:  
H319: Causes serious eye irritation.

Reagent 5:  
H290: May be corrosive to metals.  
H315: Causes skin irritation.  
H319: Causes serious eye irritation.  
H360D: H360D: May damage the unborn child.  
H410: Very toxic to aquatic life with long lasting effects.

Reagent 6:  
H302 + H332: Harmful if swallowed or if inhaled.  
H317: May cause an allergic skin reaction.  
H341: Suspected of causing genetic defects.  
H350: May cause cancer.

### Revision History

Version	Modification Comment
2024-Jul-09	Initial version with the introduction of Revision History



Consult instructions  
for use



Manufacturer



Catalog number



Batch code



Caution, consult  
accompanying documents



Use by  
YYYY-MM-DD



Temperature  
limitation

Status: 2024-Jul-09

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