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1.00362.0001

Microscopy

Silver plating kit acc. to von Kossa

for the detection of microcalcification

# For professional use only

In Vitro Diagnostic Medical Device

|  |  |
| --- | --- |
| Slide with histological specimen | |
| Distilled water | 1 min |
| Reagent 1 (Silver nitrate solution) under exposure to  illuminant | 20 min |
| Running tap water | 3 min |
| Reagent 2 (Sodium thiosulfate solution) | 5 min |
| Running tap water | 1 min |
| Nuclear fast red-aluminium sulfate solution 0.1%  (optional) | 3 min |
| Distilled water | 1 min |
| 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 e. g. Entellan® new and cover glass. | |

# Procedure

## Staining in the staining cell

Deparaffinize histological slides in the conventional manner and rehydrate

in a descending alcohol series.

The slides should be allowed to drip off well after the individual staining steps, as a measure to avoid any unnecessary cross-contamination of solu- tions.

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.

**Note:** To guarantee an optimal exposure, the distance between light source and staining cell should be about 5 cm. The light source should be placed such, that the reaction is illuminated from the top.

# Intended purpose

This “Silver plating kit acc. to von Kossa - for detection of microcalcifica- tion” 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 that when used together with other in vitro diagnostic products from our portfolio makes target structures evaluable

for diagnostic purposes (by fixing, embedding, staining, counterstaining, mounting) in histological specimen materials, for example histological sec- tions of e. g. the Mamma tissue, the heart, or the lung.

The Silver plating kit acc. to von Kossa is used to visualize calcium deposits in histological tissue specimens.

It contains two ready-to-use reagents that are needed for the silver plating. A counterstaining can be performed with, e. g. Nuclear fast red staining solution, optionally, which is not contained in the kit.

Unstained structures are relatively low in contrast and are extremely diffi- cult 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

Silver ions of the silver nitrate solution react with the carbonate and phos- phate ions of the calcium in the stored calcium deposits and displace the calcium ions.

These silver ions are reduced to metallic silver by exposure to strong light,

and this silver is evaluated by microscopy.

# Sample material

Starting materials are sections of formalin-fixed tissue embedded in paraffin (5 - 6 µm thick paraffin sections).

# Reagents

Cat. No. 1.00362.0001

Silver plating kit acc. to von Kossa

for detection of microcalcification

## Package components:

The staining kit contains

Reagent 1: Silver nitrate solution 100 ml

Reagent 2: Sodium thiosulfate solution 100 ml

**Optional** (see “Procedure”):

Cat. No. 100121 Nuclear fast red-aluminium sulfate 500 ml solution 0.1%

for microscopy

# Additionally required auxiliaries for exposure

## Also required:

Light source (e. g. desk lamp) fitted with an energy-saving lamp of at least 20 watts.

# 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 prepara-

tion. Follow the manufacturer’s instructions for application / use.

When using the corresponding auxiliary reagents, the corresponding in- structions for use must be observed.

Deparaffinize and rehydrate sections in the conventional manner.

# Reagent preparation

The reagents of the Silver plating kit acc. to von Kossa - for detection of microcalcification used for silver plating are ready-to-use, dilution of the solutions is not necessary and merely produces a deterioration of the result and their stability.

After dehydration (ascending alcohol series) and clearing with xylene or Neo-Clear®, histological samples can be mounted with water-free mounting agents (e. g. Neo-Mount®, Entellan® new, or DPX new) and a cover glass and can then be stored.

# Result

Calcium brown to black

Nuclei red

Background red

Collagen red

# Trouble-shooting

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

the procedure.

## Weak visualization of calcium deposits

* The use of a 20-Watt energy-saving lamp (with periodic exchange of the bulb), as well as the distance between the lamp and the staining cell

(5 cm, illuminated from the top) is essential.

* Further, a tissue thickness of 5 - 6 µm should be used, as mostly sections

<5 µm do not contain enough calcium material for the reaction, which

might result in false-negative results.

# Technical notes

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

When using histoprocessors or automatic staining systems, please follow

the instructions for use supplied by the supplier of the system and software.

# 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 Silver plating kit acc. to von Kossa - for detection of microcalcifi- cation at +15 °C to +25 °C.

# Shelf-life

The Silver plating kit acc. to von Kossa - for detection of microcalcification 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 +15 °C to +25 °C.

The bottles must be kept tightly closed at all times.

The used silver nitrate solution can be used for multiple reactions. However, it should be collected in a separate bottle that is then stored at 15 °C to

25 °C.

# Capacity

The package is sufficient for up to 300 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 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 dis- posal can be obtained under the Quick Link “Hints for Disposal of Microsco- py 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. 100121 Nuclear fast red-aluminum sulfate 500 ml solution 0.1 %

for microscopy

Cat. No. 100496 Formaldehyde solution 4%, 350 ml and

buffered, pH 6.9 (approx. 10% Formalin 700 ml (in

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| --- | --- | --- | --- |
|  | | solution)  for histology | bottle with wide  neck), 5 l, 10 l,  10 l Titripac® |
| Cat. No. | 100579 | DPX new  non-aqueous mounting medium for microscopy | 500 ml |
| Cat. No. | 100974 | Ethanol denatured with about 1 %  methyl ethyl ketone for analysis EMSURE® | 1 l, 2.5 l |
| Cat. No. | 103699 | Immersion oil Type N acc. to ISO 8036  for microscopy | 100-ml drop-  ping bottle |
| Cat. No. | 103999 | Formaldehyde solution min. 37%  free from acid  stabilized with about 10% methanol and  calcium carbonate for histology | 1 l, 2.5 l, 25 l |
| Cat. No. | 104699 | Immersion oil for microscopy | 100-ml drop- ping bottle, 100 ml, 500 ml |
| Cat. No. | 107960 | Entellan®  rapid mounting medium for microscopy | 500 ml |
| Cat. No. | 107961 | Entellan® new  rapid mounting medium  for microscopy | 100 ml, 500 ml,  1 l |
| Cat. No. | 108298 | Xylene (isomeric mixture)  for histology | 4 l |
| Cat. No. | 109016 | Neo-Mount®  anhydrous mounting medium for microscopy | 100-ml drop- ping bottle, 500 ml |
| Cat. No. | 109843 | Neo-Clear® (xylene substitute)  for microscopy | 5 l |
| Cat. No. | 111609 | Histosec® pastilles solidification point 56-58°C embedding agent for histology | 1 kg, 10 kg (4x  2.5 kg), 25 kg |
| Cat. No. | 115161 | Histosec® pastilles (without DMSO) solidification point 56-58°C embedding agent for histology | 10 kg (4x  2.5 kg), 25 kg |

# Main components of the products

Cat. No. 1.00362.0001

Reagent 1

AgNO3 20 g/l

Reagent 2

Na2O3S2 51.4 g/l

# Other IVD products

Cat. No. 100251 Reticulin silver plating kit 1 set

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|  | | acc. to Gordon & Sweets |  |
| Cat. No. | 100361 | ISOSLIDE® Reticulin Control Slides with reference tissue for the detection of reticular fibres in histology | 25 tests |
| Cat. No. | 100820 | Methenamine silver plating kit  acc. to Gomori for microscopy | 1 set |
| Cat. No. | 102414 | Warthin-Starry silver plating kit modified for the detection of Helicobacter pylori in paraffin sections | 1 set |
| Cat. No. | 105174 | Hematoxylin solution modified acc. to Gill III  for microscopy | 500 ml, 1 l,  2.5 l |
| Cat. No. | 109844 | Eosin Y-solution 0.5% aqueous  for microscopy | 1 l, 2.5 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.

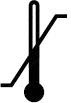
# Literature

1. Romeis - Mikroskopische Technik, Editors: Maria Mulisch, Ulrich Welsch, 2015, Springer Spektrum, 19. Auflage
2. Histotechnik, Gudrun Lang, 2013 Springer Verlag, 2. Auflage
3. Theory and Practice of Histological Techniques, John D Bancroft, Marilyn Gamble, 2008, Churchill Livingstone ELSEVIER, 6th Edition
4. Laboratory Manual of Histochemistry, Linda L. Vacca, 1985, Raven Press
5. Staining Procedures, George Clark, 1981, Williams&Wilkins, 4th Edition
6. Histological & Histochemical Methods: Theory & Practice, J. A. Kiernan, 1990, Pergamon Press, 2nd Edition
7. Histological and Histochemical Methods, Theory and practice, J. A. Kiernan, 2015, Scion Publishing Ltd, 5th Edition

Consult instructions for use

Manufacturer Catalog number Batch code

# Hazard classification

Cat. No. 1.00362.0001

Please observe the hazard classification printed on the label and the infor- mation given in the safety data sheet.

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

Caution, consult

accompanying documents

Status: 2021-Jan-18

Merck KGaA, 64271 Darmstadt, Germany, Tel. +49(0)6151 72-2440

[**www.microscopy-products.com**](http://www.microscopy-products.com/)Use by

YYYY-MM-DD

Temperature limitation

|  |  |
| --- | --- |
| **Von Kossa Staining Protocol**  Section thickness = 10μm | |
| Hydrate in distilled water | 1 min |
| Silver nitrate solution under exposure to  Illuminant (60-watt light bulb) | 120 min |
| Wash in 4x changes of distilled water | 15 seconds/wash |
| Sodium thiosulfate solution | 5 min |
| Wash in running tap water or by gently swishing slide in container of tap water. | 1 min tap water |
| Follow with 4x changes of distilled water | 15 secs/wash |
| 0.1% Nuclear fast red-aluminum sulfate solution | 45 secs |
| 1x wash in distilled water | 1 min |
| 70% Ethanol | 1 min |
| 95% Ethanol | 1 min |
| 100% Ethanol | 1 min |
| 100% Ethanol | 1 min |
| Xylene A | 15 min |
| Fresh 100% Ethanol | 1 min |
| Xylene B | 5 min |
| Mount the xylene-wet slides with DPX and cover glass. | |