1.00820.0007 REF

Microscopy

Methenamine silver plating kit acc. to Gomori

for the detection of argent-affine structures in histological tissue

For professional use only

IVD *In Vitro* Diagnostic Medical Device

CE

Intended purpose

This "Methenamine silver plating kit acc. to Gomori - for the detection of argent-affine structures in histological tissue" is used for human-medical cell diagnosis and serves the purpose of the histological investigation of sample material of human origin. It is a 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 sections of e.g. the kidney, the liver, or the lung.

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

The periodic acid Schiff (PAS) reaction is often used for the visualization of basal membranes, fungal elements, or other argent-affine structures in the tissue.
However, the Methenamine silver plating acc. to Gomori results in better and richer-in-contrast visualization of these target structures.

In the first step of the silver plating, the Periodic acid oxidizes the 1,2-glycols in the tissue to aldehydes. Due to the incubation with Methenamin-borat silver nitrate solution, these aldehydes are reduced and in turn are responsible for the reduction of the silver ions to metallic silver.
This silver will appear black.
The subsequent treatment of the tissue with gold chloride intensifies the staining of the argent-affinic structures and concurrently reduces the background staining, and the washing with sodium thiosulfate solution will remove the excess of silver-gold complexes. Like this, the target structures will exhibit a dark brown to black color and all other structures will be counterstained with light green SF.

Sample material

Starting materials are sections of tissue embedded in paraffin (3 - 5 µm thick paraffin sections).

Reagents

Cat. No. 1.00820.0007
Methenamine silver plating kit acc. to Gomori
for the detection of argent-affine structures in histological tissue

Package components:

The staining kit contains

Reagent 1:	Periodic acid solution	100 ml
Reagent 2:	Silver nitrate solution	3 x 100 ml
Reagent 3:	Methenamine borate tablets	10 pcs
Reagent 4:	Gold chloride solution	100 ml
Reagent 5:	Sodium thiosulfate solution	100 ml
Reagent 6:	Light green SF solution	100 ml

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

Preparation of the silver nitrate/methenamine borate solution

Use only freshly prepared solutions.
Dissolve 1 methenamine borate tablet (Reagent 3) in 30 ml of silver nitrate solution (Reagent 2) at room temperature.
Completely dissolve the tablet in silver nitrate solution.
The solution is now ready-to-use.

The reaction starts only once a temperature of 55 - 57 °C is achieved in the water bath; however, the solution should be used **immediately** and then discarded.

Important: Use only clean glass and plastic vessels for the preparation of silver nitrate/methenamine borate solution.
Avoid contact of metal things (e.g. slide holder tweezers) with silver nitrate/methenamine borate solution.

Procedure

Staining in the staining cell

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

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	2 min
Reagent 1 (Periodic acid solution)	10 min
Distilled water	approx. 30 sec
Distilled water	approx. 30 sec
Distilled water	approx. 30 sec
Freshly prepared Silver nitrate/methenamine borate solution at 55 - 57 °C*	35 - 45 min
Distilled water	approx. 30 sec
Distilled water	approx. 30 sec
Distilled water	approx. 30 sec
Reagent 4 (Gold chloride solution)	1 min
Distilled water	approx. 30 sec
Reagent 5 (Sodium thiosulfate solution)	2 min
Running tap water	3 min
Distilled water	approx. 30 sec
Reagent 6 (Light green SF solution)	2 - 3 min
Distilled water	approx. 30 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 e.g. Entellan™ new and cover glass.	

* Place the silver nitrate/methenamine borate solution together with the sample to be stained into the water bath previously heated to 55 - 57 °C, maintain this temperature throughout the staining process and stain for 35 - 45 minutes until achieving the desired intensity.

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.

The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

Result

Fungal elements	dark brown to black
Basal membranes	dark brown to black
Background	green

Trouble-shooting

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

Weak staining or staining with too little contrast

The color of the silver deposits can be intensified still further by treatment with gold chloride solution, formation of a silver-gold complex minimizes non-specific staining of the background. Excess silver nitrate is washed out with sodium thiosulfate solution.

Counterstaining

The counterstaining with Light green is optional. However, the cellular matrix surrounding the silver-stained structures will be visualized more clearly and more distinguishable, making diagnosis easier.

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.
Remove surplus immersion oil before filing.

Analytical performance characteristics

"Methenamine silver plating kit acc. to Gomori" 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
Detection of argent-affine structures (fungal elements and basal membranes) in histological tissue				
Fungi	12/12	12/12	8/8	8/8
Membranes	12/12	12/12	8/8	8/8
Background	12/12	12/12	8/8	8/8

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 Methenamine silver plating kit acc. to Gomori - for the detection of argent-affine structures in histological tissue at +15 °C to +25 °C.

Shelf-life

The Methenamine silver plating kit acc. to Gomori - for the detection of argent-affine structures in histological tissue 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 +15 °C.

The bottles must be kept tightly closed at all times.

The silver nitrate/methenamine borate solution should be used **immediately** and then discarded.

Capacity

The package is sufficient for up to 50 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 disposal can be obtained under the Quick Link "Hints for Disposal of Microscopy Products" at 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.	1.00579	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. 1.00820.0007

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 components of the product

Cat. No. 1.00820.0007

Reagent 1

H_5IO_6	10 g/l
1 l = 1.0 kg	

Reagent 2

$AgNO_3$	2.5 g/l
1 l = 1.0 kg	

Reagent 3

$Na_2HPO_4 \times 10\ H_2O$	23.8 g/l
$C_6H_{12}N_4$	71.40 g/l

Reagent 4

$AuCl_3$	1 g/l
1 l = 1.0 kg	

Reagent 5

$Na_2S_2O_3$	20 g/l
1 l = 1.01 kg	

Reagent 6

C.I. 42095	5 g/l
CH_3COOH	2.1 g/l
1 l = 1.0 kg	

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

H315: Causes skin irritation.
H317: May cause an allergic skin reaction.
H319: Causes serious eye irritation.
H360: May damage fertility or the unborn child.
H372: Causes damage to organs (Thyroid) through prolonged or repeated exposure if swallowed.
H410: Very toxic to aquatic life with long lasting effects.

P201: Obtain special instructions before use.
P202: Do not handle until all safety precautions have been read and understood.
P260: Do not breathe mist or vapors.
P264: Wash skin thoroughly after handling.
P270: Do not eat, drink or smoke when using this product.
P272: Contaminated work clothing should not be allowed out of the work place.
P273: Avoid release to the environment.
P280: Wear protective gloves/ protective clothing/ eye protection/ face protection.
P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308 + P313: IF exposed or concerned: Get medical advice/ attention.
P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.
P337 + P313: If eye irritation persists: Get medical advice/ attention.
P362: Take off contaminated clothing and wash it before reuse.
P391: Collect spillage.
P405: Store locked up.
P501: Dispose of contents/container to an approved waste disposal plant.

Reagent 1:
H315: Causes skin irritation.
H319: Causes serious eye irritation.
H372: Causes damage to organs (Thyroid) through prolonged or repeated exposure if swallowed.
H412: Harmful to aquatic life with long lasting effects.

Reagent 2:
H360: May damage fertility or the unborn child.
H410: Very toxic to aquatic life with long lasting effects.

Reagent 3:
H228: Flammable solid.
H317: May cause an allergic skin reaction.
H319: Causes serious eye irritation.
H360: May damage fertility or the unborn child

Revision History

Version	Modification Comment
2024-Jul-01	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-01

EMD Millipore Corporation, 400 Summit Drive, Burlington MA 01803, USA, Tel. +1-978-715-4321
MilliporeSigma Canada Ltd., 2149 Winston Park Dr, Oakville, Ontario, L6H 6J8, Canada, Phone: +1 800-565-1400
www.sigmaaldrich.com