1.02414.0001

# Microscopy

# Warthin-Starry silver plating kit modified

for the detection of Helicobacter pylori and Spirochetes in paraffin sections

**For professional use only**

IVD In Vitro Diagnostic Medical Device

## Intended purpose

This "Warthin-Starry silver plating kit modified - for the detection of Helicobacter pylori and Spirochetes in paraffin sections" 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 human-histological specimen material, for example histological sections of e.g. the stomach.

The Warthin-Starry silver plating kit modified is used to specifically detect the bacterium *Helicobacter pylori* and Spirochetes in histological specimens using 60-ml Hellendahl cells.

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

When using the Warthin-Starry silver plating, silver nitrate is reduced to metallic silver with the help of hydro quinone that is oxidized to quinone, and the development of the silver is stopped in a washing step with water. Optionally, the produced silver can be fixed and stabilized in an additional step using sodium thiosulfate solution.

When sodium thiosulfate is used, the slides can subsequently be coverslipped with any xylene-containing covering medium. In the case that this additional staining bath is dispensed with, the specimens must be mounted with DPX new or Neo-Mount® to prevent bleaching and maintain the stability of the stain color.

The reaction solution is modified in such a way that the metallic silver is deposited in the target structures in a specific reaction; in the Warthin-Starry silver plating kit modified the silver deposits on the surface of bacteria of the Helicobacter species and of the family of Spirochetes. In the microscopic picture, the bacteria will appear dark brown to black.

The bacteria will be detected in, e.g. the slime of the surface epithel, in apical stomach glands and foveoles of the stomac mucosa.

## Sample material

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

## Reagents

Cat. No. 1.02414.0001
Warthin-Starry silver plating kit modified
for the detection of Helicobacter pylori and Spirochetes in paraffin sections

**Package components:**

The staining kit contains

| | | |
|---|---|---|
| Reagent 1: | Silver nitrate solution 6 % | 500 ml |
| Reagent 2: | Hydroquinone mixture | 2x 14 g |
| Reagent 3: | Gelatine powder | 130 g |
| Reagent 4: | Acetic acid solution 1.2 % | 60 ml |

1 red dosing spoon
1 orange-colored microspoon (in the cap of the reagent 2 bottle)
1 pipette

**Note:** The contents of reagents 2 and 4 do not correspond to actual use; a residue remains in the bottle as a reserve.

**Also required:**

Cat. No. 100579 DPX new non-aqueous mounting medium for microscopy (for the use without sodium thiosulfate solution) 500 ml

or alternatively

Cat. No. 109147 Sodium thiosulfate solution $c(Na_2S_3O_3\ 5\ H_2O)$ = 0.1 mol/l (0.1 N) Titripur® Reag. Ph Eur, Reag. USP (for mounting with other xylene-containing mounting media) 1 l, 4 l Titripac®, 10 l Titripac®

Water bath
Plastic spatula

## 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 paraffin sections in the conventional manner.

## Reagent preparation

**Important:** To prepare the solutions, use only clean glass and plastic containers.

Avoid contact of solutions metal things (e.g. slide holder or tweezers).

Since the silver-plating reaction is thermosensitive, a water bath is preheated to 60 °C.

To prevent the 60-ml Hellendahl cells from cracking, they must also be preheated in the water bath.

The temperature of the reagents in the water bath is monitored using a thermometer in a 60-ml Hellendahl cell filled with distilled water.

### Distilled water

Preheat two 60-ml Hellendahl cells filled with distilled water in the water bath to a temperature of 60 °C.
These are required for the rinsing steps and can also be used for the monitoring of the temperature.

### Vinegar water (reagent 4a)

Mix 1 l distilled water with 10 ml 1.2 % acetic acid (reagent 4).

The stock solution prepared in this manner is stable for a maximum period of 3 weeks.

### Impregnation solution (reagent 1a)

To prepare approx. 60 ml of this solution, place the following substances in a preheated 60-ml Hellendahl cell and mix thoroughly by stirring with a plastic spatula:

| | |
|---|---|
| Reagent 4a (Vinegar water) | 50 ml |
| Reagent 1 (Silver nitrate solution 6 %) | 10 ml |

Place the covered solution into the 60 °C water bath at the same time as the gelatine solution (reagent 3a) and heat.

Monitor the temperature (specified temperature: 60 °C).

### Gelatine solution (reagent 3a)

To prepare approx. 60 ml of this solution, place the following substances in a preheated 60-ml Hellendahl cell and mix thoroughly by stirring with a plastic spatula:

| | |
|---|---|
| Reagent 4a (Vinegar water) | 60 ml |
| Reagent 3 (Gelatine powder) | 2 red dosing spoons |

Place the covered solution into the 60 °C water bath at the same time as the impregnation solution (reagent 1a) and heat.

Monitor the temperature (specified temperature: 60 °C).

### Developer solution (reagent 2a)

Since the developer reaction starts immediately, the developer solution must be prepared only **immediately before** the incubation of the slide in this solution.

During the rinsing step (see "Procedure", step 3) place the following substances in the 60-ml Hellendahl cell and mix thoroughly by stirring with a plastic spatula:

| | |
|---|---|
| Reagent 3a (Gelatine solution) | total quantity in 60-ml Hellendahl cell |
| Reagent 2 (Hydroquinone mixture) | 2 orange-colored microspoons (in the cap of the reagent bottle) |

Shortly before immersing the slides (see "Procedure", steps 3 and 4), add the following substances to the solution:

| | |
|---|---|
| Reagent 1a (Impregnation solution) | 3 ml (with enclosed pipette) |

and mix thoroughly in the 60-ml Hellendahl cell by stirring with a plastic spatula.

The developer solution, still at a temperature of 60 °C, is now **ready for immediate** use. The slides must be **immediately** immersed in this solution (see "Procedure", step 4), the incubation, however, can take place outside the 60 °C water bath.

**Note:** The working solutions (impregnating solution, gelatine solution, and developer solution) can be used for one experiment only and must be properly disposed of afterwards.

## Procedure

Application takes place in the 60-ml Hellendahl 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.

### without sodium thiosulfate solution

| Slide with paraffin section | | | |
|---|---|---|---|
| 1 | Distilled water | | 10 sec |
| 2 | Reagent 1a (impregnation solution) | at 60 °C | 30 min |
| 3 | strongly flowing tap water | | rinse thoroughly for 5 min |
| 4 | Reagent 2a (developer solution) | at 60 °C | 30 - 120 sec (by visual judgement) |
| 5 | Distilled water (preheated) | at 60 °C | 10 sec |
| 6 | Distilled water (preheated) | at 60 °C | 2 min |
| | Ethanol 70 % | | 30 sec |
| | Ethanol 70 % | | 30 sec |
| | Ethanol 96 % | | 30 sec |
| | Ethanol 96 % | | 30 sec |
| | Ethanol 100 % | | 30 sec |
| | Ethanol 100 % | | 2 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 DPX new and cover glass. | | | |

### with sodium thiosulfate solution

| Slide with paraffin section | | | |
|---|---|---|---|
| 1 | Distilled water | | 10 sec |
| 2 | Reagent 1a (impregnation solution) | bei 60 °C | 30 min |
| 3 | strongly flowing tap water | | rinse thoroughly for 5 min |
| 4 | Reagent 2a (developer solution) | bei 60 °C | 30 - 120 sec (by visual judgement) |
| 5 | Distilled water (preheated) | bei 60 °C | 10 sec |
| 6 | Distilled water (preheated) | bei 60 °C | 2 min |
| 7 | Sodium thiosulfate solution | | 3 min |
| 8 | Distilled water | | 10 sec |
| | Ethanol 70 % | | 30 sec |
| | Ethanol 70 % | | 30 sec |
| | Ethanol 96 % | | 30 sec |
| | Ethanol 96 % | | 30 sec |
| | Ethanol 100 % | | 30 sec |
| | Ethanol 100 % | | 2 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, DPX new, and cover glass. | | | |

After dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, histological slides can be covered with any non-aqueous mounting agents (e.g. Entellan® new, Neo-Mount®) and a cover glass and can then be stored when the silver that is formed is fixed and stabilized with sodium thiosulfate solution in an additional incubation step.

Alternatively, the specimens can exclusively be mounted with DPX new or Neo-Mount® without sodium thiosulfate fixation.

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

## Result

**without sodium thiosulfate solution:**

| | |
|---|---|
| Helicobacter pylori | dark brown to black |
| Spirochaetes | dark brown to black |
| Background | yellow to gold-yellow |

**with sodium thiosulfate solution:**

| | |
|---|---|
| Helicobacter pylori | dark brown to black |
| Spirochaetes | dark brown to black |
| Background | brownish |

## Trouble-shooting

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

**Indistinct background staining of the slide**

In the event that the slide glass appears to be contaminated, this is because silver has precipitated on the glass surface and has not been completely removed in the rinsing step (see "Procedure", step 3).
This is why care must be taken to rinse the slide intensively with tap water for 5 min.

**Bleaching of the specimens**

The silver-plated structures may gradually bleach in the event that they have been mounted with the false mounting medium.
This is why care should be taken to observe the instructions described in the specification regarding the compatibility of the xylene-containing mounting agents with or without sodium thiosulfate fixation.

**Overstaining of the specimens**

In the event that the silver-plated structures appear too black and overstained in the microscopic image, the time of incubation in reagent 2a (Developer solution) should be shortened. The stated time (30 - 120 sec, see "Procedure", step 4) should be taken as a rough guide and can thus be influenced by e. g. the thickness of the specimen section.

**Stain too weak**

- The silver-plating reaction is thermosensitive and should thus be carried out in a water bath at 60 °C. Too low temperatures will lead to poorer results. It should also be noted that the temperature indicator on the water bath in many cases is not sufficiently accurate. The temperature of the reagents in the water bath should hence be carefully monitored using a thermometer in a 60-ml Hellendahl cell filled with distilled water. **(Do not measure the temperature directly in the impregnating/gelatine/developer solutions!)**
- The rinsing steps with distilled water (see "Procedure", steps 5 and 6) should be carried out using distilled water heated to 60 °C, since otherwise the gelatines contained in reagent 2a (Developer solution) will harden on the specimen and can have a negative impact on the subsequent dehydration process.

## Technical notes

The microscope used should meet the requirements of a medical diagnostic laboratory.
When using histoprocessors and automatic staining systems, please follow the instructions for use supplied by the supplier of the system and software.
Remove surplus immersion oil before filing.

## 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 (e.g. ISOSLIDE® Warthin-Starry, Cat. No. 1.02472.0001) should be conducted with each application in order to avoid an incorrect result.

## Storage

Store the Warthin-Starry silver plating kit modified - for the detection of Helicobacter pylori and Spirochetes in paraffin sections at +15 °C to +25 °C.

## Shelf-life

The Warthin-Starry silver plating kit modified - for the detection of Helicobacter pylori and Spirochetes in paraffin sections 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 working solutions (impregnating solution, gelatine solution, and developer solution) can be used for one experiment only and must be properly disposed of afterwards.

Reagent 4a (vinegar water) is stable for a maximum period of 3 weeks.

## Capacity

The package is sufficient for up to 500 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. | 100496 | Formaldehyde solution 4%, buffered, pH 6.9 (approx. 10% Formalin solution) for histology | 350 ml and 700 ml (in 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. | 102472 | ISOSLIDE® Warthin-Starry Control Slides with reference tissue for the detection of Helicobacter pylori and Spirochetes in histological tissue | 25 tests |
| Cat. No. | 103699 | Immersion oil Type N acc. to ISO 8036 for microscopy | 100-ml dropping 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 dropping bottle, 100 ml, 500 ml |
| Cat. No. | 107164 | Paraffin pastilles solidification point about 56-58°C for histology | 10 kg (4x 2.5 kg) |
| 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 dropping bottle, 500 ml |
| Cat. No. | 109147 | Sodium thiosulfate solution $c(Na_2S_3O_3\ 5\ H_2O)$ = 0.1 mol/l (0.1 N) Titripur® Reag. Ph Eur, Reag. USP | 1 l, 4 l Titripac®, 10 l Titripac® |
| 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 |

## Hazard classification

Cat. No. 1.02414.0001

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 products

Cat. No. 1.02414.0001

Reagent 1

| | |
|---|---|
| $AgNO_3$ | 60 g/l |

1 l = 1.05 kg

Reagent 2

| | |
|---|---|
| $C_6H_6O_2$ | 42.7 wt.% |

Reagent 3

| | |
|---|---|
| Gelatine | 100.0 wt.% |

Reagent 4

| | |
|---|---|
| $C_2H_4O_2$ | ~12.6 g/l |

1 l = 1.0 kg

## Other IVD products

| | | | |
|---|---|---|---|
| Cat. No. | 100361 | ISOSLIDE® Reticulin Control Slides with reference tissue for the detection of reticular fibres in histology | 25 tests |
| Cat. No. | 100380 | ISOSLIDE® Iron Control Slides with reference tissue for the detection of free iron in histological tissue | 25 tests |
| Cat. No. | 100408 | ISOSLIDE® PAS Control Slides with reference tissue for the detection of polysaccharides in histological tissue | 25 tests |
| Cat. No. | 100425 | ISOSLIDE® Alcian blue, pH 2.5 Control Slides with reference tissue for the detection of acid mucosubstances in histological tissue | 25 tests |
| Cat. No. | 102439 | Eosin Y-solution 0.5%, alcoholic for microscopy | 500 ml, 2.5 l |
| Cat. No. | 102473 | ISOSLIDE® Methenamine Control Slides with reference tissue for the detection of argent-affine structures in histological tissue | 25 tests |
| Cat. No. | 102560 | ISOSLIDE® AFB Control Slides with reference tissue for the detection of acid-fast bacteria in histological tissue | 25 tests |
| Cat. No. | 102561 | ISOSLIDE® Congo Red Control Slides with reference tissue for the detection of amyloid structures in histological tissue | 25 tests |
| Cat. No. | 105174 | Hematoxylin solution modified acc. to Gill III for microscopy | 500 ml, 1 l, 2.5 l |
| Cat. No. | 109149 | Mayer's hemalum solution for microscopy | 500 ml, 1 l, 2.5 l |
| Cat. No. | 109204 | Giemsa's azur eosin methylene blue solution for microscopy | 100 ml, 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
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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 and Histochemical Methods, Theory and practice, J. A. Kiernan, 2015, Scion Publishing Ltd, 5th Edition



Consult instructions for use


Manufacturer

Catalog number

Batch code


Caution, consult accompanying documents


Use by YYYY-MM-DD


Temperature limitation

Status: 2021-Jan-18


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