

Warthin Starry modified for spirochetes and others microorganism technical information

Technical card code 14-132

Product code 14-132

Pack 1kit. Number of tests 100 or on request

Stability of product properly conserved at 4°C 12 months

Produce in Italy by

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in case of emergency UE number		112
in case of emergency UK number		999
en cas d'urgence Suisse		145

Purpose

The Warthin-Starry Silver-plating kit is designed for the histological detection of *Helicobacter pylori* in tissue samples using light microscopy. Principle of the method. With Warthin-Starry silver stain, silver nitrate is reduced to metallic silver by means of hydroquinone; hydroquinone is thereby oxidized into quinone. The reaction solution is selected so that the metallic silver is added to a reaction specific to the target structures, in this case *Helicobacter pylori*. The reaction is stopped by a rinsing step, which ends the silver development. Silver is stored on the bacteria surface; the bacteria appear dark brown to black in the microscopic image; cells are yellow to gold yellow; the nuclei are brown. The bacteria are found in the mucus of the surface epithelium, in the apical gastric glands and in the foveolae of the gastric mucosa.

Material

Tissue samples fixed with neutral buffered formalin.

3 5 µm thick paraffin sections Avoid the use of metallic object.

Method

time	temperature	step
Deparaffinise and rehydrate sections in the usual manner.		
10 second		distilled water
30 minutes	60°C	impregnation solution
3 minutes		rinse well in running tap water
2-3 minutes	60°C	developer solution (see statement)
10 second	60°C	distilled water
2 minutes	60°C	distilled water
Dehydrate and xylene mount with DdMount		

Result:

<i>Helicobacter pylori</i>	dark brown to black
Background	yellow to gold-yellow

Impregnation solution preparation

To 50 ml of acidulated water add 10 ml of silver nitrate 6% solution, stir and place covered in the hot water bath and heat up to 60°C. Check of temperature (solution is ready-to-use). Important do not use any metal things

Developing solution

To 60 ml of acidulated water add 2 gr of gelatine and place covered in the hot water bath at the same time as the impregnation solution, and stir occasionally. The gelatine must be completely dissolved. Further additions just prior to place the sections into this solution. (see finishing)

Finishing

Add 2 gr of hydroquinone and stir well. Add 3 ml hot impregnation solution with a pipette, mix well.

Note

The sections must be placed immediately into the ready-to-use developer solution!

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Solutions

Acidic water	3x100 ml
Silver nitrate impregnation 6 %	2x30 ml
Jelly	6 gr
Hydroquinone for development	6 gr

* Notes.

Distilled water or tap water can be used for rinsing and moisturizing.

Always check the pH of your tap water and chlorine levels before proceeding with any type of biological tissue and stain.

* Technical's note: staining time vary according to age, types of solutions, thickness of sections, et. When Gill (code 09-178) modified solution is used, get the best result, staining time (maximum 1-5 minutes), for best change in color, wash quickly in tap water, and then in Scott acidulated solution, (code 00-136). For sections fixed in Bouin, we recommend the use of haematoxylin modified acid AB (code 09-183). Please note the alcoholic loses eosin stain with the use, of the days are stretched over time colouring. If you are using purified eosin, check the time, and possibly diluted in ethyl alcohol 96°C, if the cytoplasmic staining was too strong.

* Before use, filter the following solutions; alcoholic eosin, eosin phloxine; Harris haematoxylin, Gill's haematoxylin. The acidified aqueous solution of eosin is prepared by slowly adding glacial acetic acid.

* Follow normal precautions for laboratory reagents. Dispose of waste according to regulations at the local, regional or national level. Refer to Data Sheet Material Safety Data for updated information on risks, hazards and safety associated with the use of these products.

* Risk and Safety Statements outside the EU.

The eosin solution in alcohol is flammable and harmful. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).

* Target organs: eyes and nerves.

Eosin in aqueous solution. Caution: substance not yet fully tested. Avoid contact and inhalation of the solution of Harris haematoxylin. Organs: heart and nerves. Solutions based hemallum are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing. Organs affected: liver and kidneys.

In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).

* Risk and Safety Statements (U.E.)

The eosin solution in alcohol is highly flammable and harmful. Highly flammable. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible). Eosin in aqueous solution.

Caution: Substance not yet fully tested. Solution of hemallum. Do not breathe vapors. Avoid contact with skin and eyes. Gill haematoxylin Solutions are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing.

* The microscope used should meet the requirements of a medical diagnostic laboratory. Carefully follow the instructions for the fixative. If an automated tool was used for staining, follow the instructions of the equipment and software.

Remove surplus immersion oil before storing.

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* Sample preparation

All samples must be treated according to the technology. All samples must be marked so as to be easily identified. Tools should be used for sampling and sample preparation, which must be observed strictly to manufacturer's instructions about the application and instructions.

* Diagnostics

The diagnosis should be performed only by authorized and trained persons. Valid nomenclatures must be used. Further tests must be selected and implemented according to recognized methods.

* Conservation. The staining solution should be stored at a temperature between +15°C to 20°C, the dye at +5°C to 30° C. Store at 4-6 °C all kit containing silver solutions and Schiff reagents. The solution and dyes must be used before the expiration date. Stability. After first opening the bottle, the dye solution and the dyes are stable until the expiration date when stored at the temperature requested. Always keep the bottles tightly closed.

* Instructions for use

To avoid errors, the staining process must be performed by qualified personnel. For professional use only. Must observe the National guidelines for work safety and quality assurance. Microscopes are used according to the standard. Protection against infection. Must be taken with laboratory guidelines for the protection against infection.

* Instructions for disposal

The solutions used and those have expired must be disposed of as special waste according to local regulations regarding disposal of waste.

Endnotes

1 The timing suggested in the leaflet are approximate and may vary according to your specific needs. If they are used intensively, for staining solutions may lose their dyes, so it is necessary to extend the time of staining solutions, or replace with new products.

2. Include positive control slides in each session.

3. Some hydraulic systems deliver acidic water, unsuitable for use for the part of the procedure for the blue coloration. If tap water is acidic, instead using a dilute alkaline solution, for example, water buffered by Scott.

4. The presence of purple or red-brown nuclei a blue color indicates unsatisfactory.

5. If you over-eosin staining, nuclear staining may be masked. If done correctly, with eosin staining shows a three-tone effect. To increase the differentiation of eosin, extend the time of immersion in alcohol, or use a first alcohol with a higher water content. You can adjust the times of immersion in alcohol to obtain an adequate eosin staining.

6. We do not recommend the addition of stock solution in the working solutions of haematoxylin and eosin.

7. Avoid excessive drag (carryover) of water solutions in alcoholic eosin.

8. The data generated by this procedure are to be used only to support the diagnosis and should be evaluated in conjunction with other tests and diagnostic data

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