Warthin Starry Stain Kit

Laboratory Use Only

PRODUCT INFORMATION:

REF

SSP014 100ml
SSP014 250ml
SSP014 500ml

PERFORMANCE CHARACTERISTICS:

Staining Interpretation
Helicobacter pylori : Black
Other tissue elements : Pale yellow

SUMMARY AND EXPLANATION

For *laboratory* use only

The Warthin-Starry Stain Kit is intended for use in the visualization of Spirochetes, Helicobacter pylori, Helicobacter pylori is a Gram negative, spiral organism, which colonizes the gastric mucosa. To enhance the detection of the presence of low-density organism requires special staining techniques such as Warthin-Starry stain. From a practical point of view, the identification is relatively much easier with the Warthin-Starry stain because the silver coating makes the organism appear larger. This product is not intended for diagnostic or therapeutic use The results are to be interpreted by qualified personnel in conjunction with other clinical and laboratory findings.

PRINCIPLE OF THE PROCEDURE

The Warthin-Starry silver stain is used to specifically detect the bacterium Helicobacter pylori in histological specimens of human origin. The bacteria are found in the mucus of the surface epithelium, in the apical gastric glands and in the venules of the gastric mucosa. Warthin starry relies on the ability of certain microorganisms to bind silver ions from the solution. The method is based on silver impregnation. With Warthin-Starry, the H.pylori were visualized not only on the surface of the foveolar epithelium but also deep inside the gastric pits.

The first stage of the procedure involves the impregnation of clusters of silver atoms on the organism. Slides are then treated with a reducing solution that typically contains hydroquinone, gelatin, and a lower concentration of silver nitrate. This solution acts as a type of "developer": The gelatin sequesters silver ions, slowing the rate at which they are reduced to metal by the hydroquinone. After treatment with Developer, a reducing agent, impregnated silver is converted into metallic silver, resulting in a dark brown appearance. The tissue is then counterstained with Tartrazine, which gives a pale yellow colour where the organism appears black.

REAGENTS PROVIDED

Kit Components	Product Code	Storage conditions	Pack Sizes		
			100ml	250ml	500ml
Acidulated Silver Nitrate Solution - A (Reagent A)	IPS064	2-8º C	250ml	320ml*2	450ml*3
Acidulated Silver Nitrate Solution - B (Reagent B)	IPS065	2-8º C	25ml	65ml	130ml
Acidulated Gelatin (Reagent C)	IPS066	2-8º C	75ml	200ml	400ml
Acidulated Hydroquinone (Reagent D)	IPS072	2-8º C	25ml	65ml	130ml
Tartrazine Solution (Reagent E)	IPS068	RT	100ml	250ml	500ml

STORAGE AND HANDLING

Storage Recommendations: Store at the recommended storage conditions specified for each reagent. When stored at the appropriate conditions, the reagents are stable until expiry. **Do not use the reagents after the expiration date provided on the vial.**

To ensure proper regent performance, delivery, and stability, replace the dispenser cap after each use and immediately place the vials at the recommended temperature, away from sunlight, in an upright position.

During transport, short-term exposure to 2-8°C does not affect product performance.

SPECIMEN PREPARATION

Sample preparation and fixation: Formalin-fixed, Paraffin-embedded tissue sections of 3- 5 µm thickness on microscopic slides

PRECAUTIONS

1. Normal precautions exercised in handling laboratory reagents should be followed.
2. This product should be used by qualified and trained professional users only
3. It can cause serious eye and skin irritation. Refer to Material Safety Datasheet for any updated risk, hazard or safety information.
4. Dispose of waste observing all local, state, provincial or national regulations.
5. Do not use reagents after expiration date
6. Use protective clothing and gloves, while handling reagents
7. Avoid contamination of reagents as it may lead to incorrect results
8. Place the reagents back at the recommended storage conditions upon usage
9. Use dust-free and cleaned plastic/glassware. Any particulate or ionic contamination can introduce debri on slides

MATERIALS REQUIRED, BUT NOT PROVIDED

- Xylenes
- Graded alcohols (50%, 70%, 95%, Absolute)
- DPX Mountant
- Microscopic slides (Positively charged)
- Hot air oven
- Slide holder
- Jars
- Cover slips
- Coplin jars

PREPARATION OF WORKING SOLUTION

Developer Solution:

Prepare the Developer Solution freshly in a clean, unused conical centrifuge tube (wrapped in foil) just before use. Prepare the developer solution in the following order and make sure that Acidulated Gelatin (Reagent C) is pre-heated to 60°C

Combine: 2ml Acidulated Hydroquinone (Reagent D)
6 ml pre-heated Acidulated Gelatin (Reagent C)
2ml Acidulated Silver Nitrate Solution - B (Reagent B)

(It can be used for approximately 8-10 slides. Mix the above amounts (or the required proportions thereof) thoroughly.

Note: Do not reuse the developer. Once the solution turns dark or cloudy, it must be discarded. The solution cannot be reused.

STAINING PROCEDURE

Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place the slide in 30ml of Acidulated Silver Nitrate Solution – A (Reagent A) in a plastic Coplin jar. Ensure that the solution is preheated to 60 °C in a Water bath. Let the slide stand for 5-7 minutes to allow the silver impregnation to occur in the solution at 60 °C.
3. Prepare the Developer working solution just before the addition (Refer to the working solution Preparation above).
4. Remove the slide from Acidulated Silver Nitrate Solution – A (Reagent A) and treat the sections with the freshly prepared heated Developer solution. (Note: The tissue should not get dried before placing it in the developer solution. Drying causes silver to precipitate unevenly, resulting in artifacts and poor contrast)
5. Allow the slide to remain in the developer solution until the sections appear grey or brown at room temperature. This usually takes about 1-10 minutes but may take longer. (Cover the lid during this step)
   (Monitor slides microscopically during development. An ideal endpoint is when sections turn gray-brown)
6. Wash quickly and thoroughly under hot tap water (60ºC) for 5 minutes (Use generous and repeated washing steps after developer application. Improper washing might lead to background staining as Gastric tissues require particular care, as mucous content retains excess silver. Inadequate washing in these samples leads to excessive background staining)
7. Rinse in two changes of distilled water for 1 minute each

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8. Counterstain with Tartrazine Solution (Reagent E) for 1-2 minutes.
9. Quickly rinse in distilled water (1 dip) and make sure to air dry.
10. Quickly dehydrate (1 dip) in graded alcohols (90%, 100%, 100%, 100%, 100%)
11. Clear in three changes of xylene.
12. Mount with compatible mounting media and coverslip.

QUALITY CONTROL

The recommended positive control for the Warthin Starry Stain Kit is Helicobacter pylori-infected tissue.

PERFORMANCE CHARACTERISTICS

Warthin Starry Stain Kit stains Helicobacter **pylori** in **Black** colour, **Nuclei** in **Brown** colour and **other tissue elements** in **Pale Yellow** colour.

TROUBLESHOOTING

1. Follow the specific protocol recommendations according to the data sheet provided.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, reagent trapping or inaccurate results.
3. Do not allow the section to dry out during the entire staining process.
4. Gently mix all the reagents prior to use.
5. Excessive or incomplete counterstaining may compromise the interpretation of the results.
6. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 or E-mail: techsupport@pathnsitu.com

LIMITATIONS AND WARRANTY

1. This product is intended for use only by authorised, trained, and qualified personnel.
2. A qualified and trained pathologist/personnel must interpret the results of the test.
3. Interpretation of test results must be made in conjunction with relevant background information and additional laboratory findings.
4. Always use the recommended volume and concentration of reagents to ensure complete coverage of the tissue section and to minimise the risk of false-positive or false-negative results.
5. Use appropriate buffers, instruments, consumables, and incubation conditions as recommended to achieve optimal staining performance.
6. It is strongly recommended to include known positive and negative controls when performing the test to ensure the validity of results.
7. The product has been validated on formalin-fixed, paraffin-embedded (FFPE) tissues. The end user must establish performance on other tissue types.
8. Unexpected results may occur in untested tissues due to inherent variability in tissue components.
9. False-positive reactions may occur due to insufficient washing, inappropriate protocol conditions, or other contributing factors.
10. In instances where the staining pattern or localisation differs from the specifications outlined in this datasheet, please get in touch with technical support for guidance.
11. Maintain the product under the recommended storage conditions to preserve reagent stability and performance.
12. Do not use reagents that appear cloudy, discoloured, or show signs of contamination. Discard any components showing signs of deterioration.
13. Silver Nitrate is light sensitive. Cover containers during use. Avoid exposing silver nitrate to bright light, including direct sunlight, as this can cause the chemical to decompose.
14. Gastric tissues contain mucus, which easily traps silver. If not thoroughly washed, excess silver binds non-specifically, leading to a dirty background.
15. This product is intended for single-use application only. Once applied to a tissue section, reagents should not be recovered or reused, as this may compromise test integrity and specificity.
16. PathnSitu makes no warranties beyond those expressly stated in the product description.
17. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
18. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
19. For detailed information and specifications on individual components, please refer to the Product Material Safety Data Sheet (MSDS)

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BIBLIOGRAPHY

1. Histochemical and Immuno Histochemical methods for the detection of Spirochetes in the biopsies of skin; Zanconati, Cattonar, Grandi
2. Silver staining for Spirochetes in tissues: Rationale, Difficulties, Troubleshooting; J. A. Kiernan
3. Identification of Helicobacter pylori by different conventional staining techniques and its comparison with polymerase chain reaction; Article in Saudi medical journal · September 2013
4. Handbook of histological and special staining techniques of the Armed Forces Institute of Pathology.

EXPLANATION OF SYMBOLS

LOT	Lot number / Batch number		Expiry
°C °C	Storage limitation	RT	Room Temperature
	Date of manufacture	REF	Catalogue number
	Manufacturer address		

US Office: 7026 Koll Center Parkway, STE#211, Pleasanton, CA-94566, USA Ph: +1 925-218-6939,
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