

Warthin Starry Stain

PRODUCT INFORMATION:

SSP014 100ml Ready to use
 SSP014 250ml Ready to use
 SSP014 500ml Ready to use

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, Legionella pneumophila, and Cat Scratch Fever bacteria. 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. The stain is widely used in histological applications for both diagnostic and research purpose.

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 veolae 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 the 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 the treatment with Developer, a reducing agent, impregnated silver is converted in to metallic silver which gives dark brown appearance. The tissue is then counter stained with Tartrazine which gives pale yellow color where the organism appears black in color.

REAGENTS PROVIDED

Kit Components	Product Code	Storage conditions	Pack Sizes		
			100ml	250ml	500ml
0.25% Silver Nitrate (Acidulated) Reagent A	IPS064	2-8° C	250ml	320ml*2	450ml*3
2% Silver Nitrate (Acidulated) Reagent B	IPS065	2-8° C	25ml	65ml	130ml
Gelatin (Acidulated) Reagent C	IPS066	2-8° C	75ml	200ml	400ml
Hydroquinone (Acidulated) Reagent D	IPS072	2-8° C	25ml	65ml	130ml
Tartrazine Reagent E	IPS068	RT	100ml	250ml	500ml

STORAGE AND HANDLING

Storage Recommendations: Store at appropriate storage conditions of respective reagents. When stored at the appropriate conditions, the reagents are stable until expiry. **Do not use the reagents after expiration date provided on the vial.** To ensure proper reagent delivery and stability, replace the dispenser cap after every use and immediately place the vials at recommended temperatures away from sunlight in an upright position.

SPECIMEN PREPARATION

Recommended positive controls: Helicobacter pylori infected tissue
Sample preparation and fixation: Formalin-fixed, Paraffin-embedded tissue sections of 3- 5 µm thickness on microscopic slides
Reagent Preparation: Make sure all the reagents are at room temperature before the preparation and place them back at recommended storage conditions after use.
Developer Solution: Freshly prepare the Developer Solution in an unused clean conical centrifuge tube (wrapped in foil) just before use. Prepare developer solution in the following order and make sure that acidulated Gelatin (Reagent C) is pre-heated to 60°C

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

(It can be used for approximately 8-10 slides. Mix the above amounts (or needed proportions thereof) well. The solution cannot be reused)

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 microbial contamination of reagents as it may lead to incorrect results
8. Place the reagents back at the recommended storage conditions upon usage

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

STAINING PROCEDURE

Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place the slide in 30ml of 0.25% Silver Nitrate Solution (Reagent A) in a plastic coplin jar. Make sure that the solution is pre-heated to 60° C in Water bath. Let the slide stand for 5-7 minutes for the silver impregnation in the solution at room temperature.
3. Prepare the Developer working solution just before the addition (Refer to Reagent Preparation above).
4. Remove the slide from 0.25% Silver Nitrate solution (Reagent A) and treat the sections with the freshly prepared heated Developer solution. (Note: The tissue should not get dried before placing in the developer solution)
5. Allow the slide to remain in the developer solution until the sections appear gray-brown. This usually takes about 1-10 minutes but may take longer. (Cover the lid during this step)
6. Wash quickly and thoroughly under hot tap water (60° C) for 5 minutes (Improper washing might lead to back ground staining due to silver nitrate)
7. Rinse in two changes of distilled water for 1 minute each
8. Counter stain with 0.1% 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%)
11. Clear in three changes of xylene.
12. Mount with DPX and coverslip.

QUALITY CONTROL

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

PERFORMANCE CHARACTERISTICS

Warthin-Starry Stain for Helicobacter pylori stains Black color, Nuclei stains Brown color and other tissue elements stains Pale Yellow color.

TROUBLESHOOTING

1. Follow the specific protocol recommendations according to 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. Excessive or incomplete counterstaining may compromise the interpretation of the results
5. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 or E-mail: techsupport@pathnsitu.com

LIMITATIONS AND WARRANTY

Authorized and skilled personnel only may use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results. A qualified pathologist must perform the evaluation of the test results. There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable for property damage, personal injury, time or effort on economic loss caused by this product.

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. Hand book of histologic and special staining techniques of the Armed Forces Institute of Pathology.

EXPLANATION OF SYMBOLS

 Lot number / Batch number



Expiry



Storage limitation

Laboratory use only

RT- Room Temperature