PathnSitu Biotechnologies

Laboratory Use Only

Acid-Fast Bacteria (AFB-NA) Stain
(Green Counterstain)

PRODUCT INFORMATION: **PERFORMANCE CHARACTERISTICS:**

REF

SSP017-NA 100ml
SSP017-NA 250ml
SSP017-NA 500ml

Staining Interpretation:
Acid Fast Bacilli : Bright Red
other tissue elements: Pale green

SUMMARY AND EXPLANATION

For *laboratory* use only

Acid-Fast Bacteria (AFB-NA) Stain Kit (Green Counterstain) is a differential bacteriological stain used to identify and detect the presence of acid-fast Mycobacteria in FFPE tissues. Acid-fast techniques are valuable for detecting mycobacteria, rod-shaped organisms that sometimes exhibit filamentous (fungus-like) growth. The most significant disease-causing mycobacteria are Mycobacterium tuberculosis and Mycobacterium leprae. The staining method for acid-fast bacilli is like the classical bacteriological procedure for smears. This product is not intended for diagnostic or therapeutic use. The results should be interpreted by qualified personnel in conjunction with other clinical and laboratory findings.

PRINCIPLE OF THE PROCEDURE

The term "acid fast" describes the ability of certain bacteria to bind cationic dyes and retain them after differentiation in an acidic solution. Typically, cationic dyes like basic fuchsin are dissolved in an aqueous solution containing lipophilic agents such as alcohol and phenol. Alcohol increases the solubility of the dye molecules, while phenol helps the dye penetrate the waxy capsule of acid-fast bacteria. After staining with the dye, the specimens are treated with an acidic solution; only the acid-fast bacteria retain the stain, as other bacteria are decolorized. Remaining cells and tissue components are counterstained with Light Green.

REAGENTS PROVIDED

Kit Contents	Product Code	Storage Conditions	Pack Sizes		
			100ml	250ml	500ml
Carbol Fuchsin Solution (Reagent –A)	IPS054	RT	100ml	250ml	500ml
Decolorizer Solution (Reagent B)	Not Provided- Refer to Reagent Preparation				
Light Green Solution - C (Reagent C)	IPS056	RT	100ml	250ml	500ml

STORAGE AND HANDLING

Storage Recommendations: Store at Room temperature. 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 reagent performance 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 temperatures between 2-8 °C does not affect product performance.

SPECIMEN PREPARATION

RECOMMENDED POSITIVE CONTROLS:

1. Acid-fast bacilli infected tissue

SAMPLE PREPARATION AND FIXATION:

- Formalin-fixed, Paraffin-embedded tissue sections of 3- 5 µm thickness on microscopic slides

PRECAUTIONS

1. Normal precautions should be followed when handling laboratory reagents.
2. This product should only be used by qualified and trained professionals.
3. It can cause serious eye and skin irritation. Refer to the Material Safety Data Sheet for any updated risk, hazard, or safety information.
4. Dispose of waste in accordance with all local, state, provincial, or national regulations.
5. Do not use reagents after the expiration date.
6. Wear protective clothing, gloves, and a face mask when handling reagents.
7. Avoid contaminating reagents, as it may lead to inaccurate results.

MATERIALS REQUIRED. BUT NOT PROVIDED

- Xylenes
- Graded alcohols (50%, 70%, 95%, absolute)
- 1% Acid Alcohol
- DPX Mountant
- Microscopic slides (positively charged)
- Slide holder
- Jars
- Cover slips
- Coplin jars
- Distilled water
- Water bath/ Spirit lamp
- Filter paper

PREPARATION OF WORKING SOLUTION

Refer to the pack size (listed on the box and empty labelled bottle) that was received before making any working solutions.

- Prepare it into an empty labelled bottle provided in the kit

Preparation of Decolorizer Solution

Reagent	Quantity Required		
	100ml	250ml	500ml
70% Alcohol	99ml	247.5	495ml
Concentrated Hydrochloric Acid (HCl)	1ml	2.5ml	5ml

Note: Once the stock reagents are prepared, they remain stable until the expiration date of the kit.

STAINING PROCEDURE

Pre-staining Preparation:

Filter Reagent A: Filter Carbol Fuchsin Solution using Filter Paper whenever a thick sheen develops on the solution surface.

Protocol (I) : (Conventional Method)

1. Deparaffinize and rehydrate the tissue sections.
2. Stain the sections with Carbol Fuchsin Solution (Reagent A) for 20 minutes at 60°C using a water bath.
3. Wash the slides running tap water for 2-3 minutes
4. Differentiate in Decolourizer Solution (Reagent B) (Refer to the Reagent Preparation) until the tissue section turns pale pink or no colour.
5. Wash in running tap water for 3 minutes; rinse in 2 changes of distilled water.
6. Counterstain in Light green Solution – C (Reagent C) for a few seconds. Quickly wash off the counter stain after 3-5 seconds.
   Note: (Overstaining may mask the staining of bacteria.)
7. Quickly rinse in distilled water.
8. Dehydrate quickly in two changes of 95% and 100% Alcohol. Clear in three changes of xylene,10 dips each
9. Cover slips with compatible mounting medium.

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Protocol (II): (Heat Fixation Method)

1. Deparaffinize and rehydrate the tissue sections.
2. Stain the sections with Carbol Fuchsin Solution (Reagent A). Intermittently, heat the slide with a spirit lamp for 5 minutes. Allow the slides with Carbol Fuchsin to stand at room temperature for 5 minutes.
3. Wash the slides under running tap water for 2-3 minutes.
4. Differentiate in Decolourizer Solution (Reagent B) (refer to the Reagent Preparation) until the tissue section turns pale pink or no colour remains.
5. Wash in running tap water for 3 minutes; rinse in 2 changes of distilled water.
6. Counterstain in Light Green Solution – C (Reagent C) for a few seconds. Quickly wash off the counterstain after 3-5 seconds.
7. Note: (Overstaining may mask the staining of bacteria.)
8. Rinse quickly in distilled water.
9. Dehydrate rapidly in two changes of 95% and 100% alcohol. Clear in three changes of xylene, with 10 dips each.
10. Cover the slide with a compatible mounting medium.

QUALITY CONTROL

The recommended positive tissue control for the Acid-Fast Bacteria (AFB-NA) Stain Kit (Green Counterstain) is AFB-infected tissue.

PERFORMANCE CHARACTERISTICS

Acid-Fast Bacteria (AFB-NA) Stain Kit (Green Counterstain) stains **acid-fast bacilli** in **bright red** and **other tissue elements** in **pale green.**

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. 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. 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.
14. Ideally, perform staining in a chemical fume hood. Never leave slides unattended while heating them during Carbol fuchsin incubation. Wear a face mask and avoid inhaling vapours released during heating.
15. PathnSitu makes no warranties beyond those expressly stated in the product description.
16. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
17. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
18. For detailed information and specifications on individual components, please refer to the Product Material Safety Data Sheet (MSDS)

BIBLIOGRAPHY

1. Demonstration of Acid fast bacilli in Tissue Sections* EL W. WADE, MD.
2. Manual of Histologic and Special staining Techniques: Armed Forces Institute of Pathology
3. Acid fast stains Protocols; American Society for Microbiology ; Marise A. Hussey • Anne Zayaitz

EXPLANATION OF SYMBOLS

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

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