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

PRODUCT INFORMATION: **PERFORMANCE CHARACTERISTICS:**

REF

SSP012 100ml Ready to use **Staining Interpretation:**
SSP012 250ml Ready to use **Acid Fast Bacilli :** Bright Red
SSP012 500ml Ready to use **Other tissue elements :** Pale green

SUMMARY AND EXPLANATION

For *laboratory* use only

Acid-Fast Bacteria (AFB) 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 of value in the detection of mycobacteria, rod-shaped organisms that sometimes exhibit filamentous (fungus-like) growth. The most significant disease-producing mycobacteria are Mycobacterium tuberculosis and Mycobacterium leprae. The staining method for acid-fast bacilli is like that of a classical bacteriological procedure for smears. 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 term "acid-fast" refers to the capacity of specific bacterial types to bind cationic dyes and to retain these dyes following differentiation in an acidic solution. Typically, cationic dyes, such as basic fuchsin, are incorporated into an aqueous solution containing lipophilic compounds, including alcohol and phenols. The alcohol increases the solubility of the dye molecules, and the phenol facilitates the movement of the dye molecules through the waxy capsule of the acid-fast bacteria. Following staining with the dye, the specimens are differentiated in an acidic solution, and only the acid-fast bacteria retain the stain, as other bacteria undergo decolourization. Other cell 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)	IPS055	RT	100ml	250ml	500ml
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 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 temperatures between 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 carried out in handling laboratory reagents should be followed
2. This product should be used by qualified and trained professional users only

Laboratory Use Only

3. The product contains alcohol and is classified as highly flammable; it must be kept away from ignition sources
4. It can cause serious eye and skin irritation. Refer to Material Safety Datasheet for any updated risk, hazard or safety information
5. Dispose of waste, observing all local, state, provincial or national regulations
6. Do not use reagents after the expiration date
7. Use protective clothing and gloves while handling reagents
8. Avoid contamination of reagents, as it may lead to incorrect results

MATERIALS REQUIRED. BUT NOT PROVIDED

- Xylenes
- Graded alcohols (50%, 70%, 95%, Absolute)
- DPX Mountant
- Microscopic slides (Positively charged)
- Slide holder
- Jars
- Cover slips
- Coplin jars
- Filter Paper
- Water bath/Spirit lamp
- Distilled water

STAINING PROCEDURE

Pre-staining Preparation:

Filter Carbol Fuchsin solution (Reagent A) 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. Rinse in running tap water for 2-3 minutes.
4. Differentiate in Decolouriser Solution (Reagent B) until the colour no longer runs off the slide and sections turn from 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. Wash off the counter stain quickly after adding it.
   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 (E.g, DPX)

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 using 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) until the colour no longer runs off the slide and the sections are pale pink.
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. Wash off the counter stain quickly after adding it.
   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 slip using compatible mounting medium (E.g.DPX).

QUALITY CONTROL

The recommended positive tissue controls for the Acid-Fast Bacteria Stain Kit (Green Counterstain) are tissues infected with acid-fast bacilli.

PERFORMANCE CHARACTERISTICS

Acid-Fast Bacteria (AFB) Stain Kit (Green Counterstain) stains **Acid Fast bacilli** in **Bright Red** and **other tissue elements** in **Pale green** in 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. 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 number / Batch number

Expiry

Storage limitation

RT **Room Temperature**

Date of manufacture

Catalogue number

Manufacturer address