Acid Fast Bacteria Stain (Green Counterstain)

Laboratory Use Only CE

PRODUCT INFORMATION:
SSP012 100ml Ready to use
SSP012 250ml Ready to use
SSP012 500ml Ready to use

PERFORMANCE CHARACTERISTICS:
Staining Interpretation:
Acid Fast Bacilli : Bright Red
Other tissue elements : Pale green

SUMMARY AND EXPLANATION

For *laboratory use* only

AFB stain is a differential bacteriological stain used to identify acid-fast organisms, mainly Mycobacterium species including M.tuberculosis, M.ulcerans, and M.leprae and non-tuberculous mycobacteria (NTM).The staining method for acid fast bacilli is similar to that of classical bacteriological procedure for smears. The Acid Fast Stain Kit when used in the appropriate histological procedures may be used for the detection of Mycobacterium tuberculosis in tissue sections and smears.

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 factors such as alcohol and phenol. 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 decolorization. Other cells / tissue components are counter stained with light green.

REAGENTS PROVIDED

Kit Contents	Product Code	Storage Conditions	Pack Sizes		
			100ml	250ml	500ml
Carbol Fuchsin (Reagent A)	IPS054	RT	100ml	250ml	500ml
Decolorizer (Reagent B)	IPS055	RT	100ml	250ml	500ml
Light Green (Reagent C)	IPS056	RT	100ml	250ml	500ml

STORAGE AND HANDLING

Storage Recommendations: Store at recommended temperatures. 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 regent 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: 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 exercised in handling laboratory reagents should be followed
2. This product should be used by qualified and trained professional users only
3. The product contains alcohol and is classified as highly-flammable, 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 expiration date
7. Use protective clothing and gloves, while handling reagents
8. Avoid microbial 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
- Hot air oven
- Cover slips
- Coplin jars

STAINING PROCEDURE

Pre-staining Preparation:

Filter Carbol Fuchsin (Reagent A) using filter paper whenever a thick sheen develops on solution surface.

Protocol (I): (Conventional Method)

1. Deparaffinize and rehydrate the tissue sections.
2. Stain the sections with Carbol Fuchsin (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 Decolorizer (Reagent B) until the color no longer runs off the slide and sections turns from pale pink or no colour.
5. Wash in running tap water for 3 minutes; rinse in 2 changes of distilled water.
6. Counter stain in Light green (Reagent C); for few seconds. Quickly wash off the counter stain soon after adding it.
   Note: (Over staining may mask the staining of bacteria)
7. Quick 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 with compatible mounting medium.

Protocol (II): (Heat Fixation Method)

1. Deparaffinize and rehydrate the tissue sections.
2. Stain the sections with Carbol Fuchsin (Reagent A). Intermittently, heat the slide using a spirit lamp for 5 minutes. Allow the slides with Carbol Fuchsin to stand for 5 minutes at room temperature.
3. Wash the slides under running tap water for 2-3 minutes
4. Differentiate in Decolorizer (Reagent B), until color no longer runs off the slide and sections are pale pink.
5. Wash in running tap water for 3 minutes; rinse in 2 changes of distilled water.
6. Counter stain in Light Green (Reagent C) for few seconds. Quickly wash off the counter stain soon after adding it.
   Note: (Over staining may mask the staining of bacteria)
7. Quick 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 DPX.

PERFORMANCE CHARACTERISTICS

AFB for Acid Fast bacilli stains Bright Red in color and other tissue elements stains Pale Green in 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

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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

RT- Room Temperature

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