PAS-Diastase (PAD) Stain Kit

PRODUCT INFORMATION:

REF

SSP006 100ml Ready to use
SSP006 250ml Ready to use
SSP006 500ml Ready to use

PERFORMANCE CHARACTERISTICS:

Staining Interpretation:
Mucins : Magenta
Nucleus : Purple or Dark Blue
Glycogen : Digested and not stained

SUMMARY AND EXPLANATION

For *laboratory* use only

PAS-Diastase (PAD) Stain Kit refers to the PAS stain used in combination with diastase enzyme to differentiate glycogen from PAS-positive elements in tissue samples. The PAS-D method is also used for periportal liver staining of AAT polymer inclusions that are seen in alpha-1 antitrypsin deficiency disease. The PAS with Diastase staining procedure can also be used to differentiate glycogen granules from other granules in various tumor types.

Mucin can be identified explicitly in specific tissue samples using the PAS staining procedure only if the glycogen (which is also PAS-positive) is digested with diastases and washed out. In cirrhosis, α_1-AT globules characteristically occur at the periphery of the nodules in multiple sizes within the hepatocyte, giving a dark, reddish-purple colour when stained with PAS-diastase, as glycogen is digested by diastase.

PRINCIPLE OF THE PROCEDURE

Diastase, also known as Alpha-Amylase, is an enzyme commonly present in saliva. Alpha-Amylase degrades glycogen into a mixture of water-soluble sugars, disaccharide maltose, trisaccharide maltotriose and dextrins by cleaving the α-glucosidic 1,4 linkages. These water-soluble sugars are then washed from the section.

The periodic acid acts as an oxidising agent, which oxidises compounds having free hydroxyl groups or amino/alkylamine groups. The tissue sections are first oxidised using periodic acid, which oxidises the vicinal bonds in these sugars, breaking the carbon-carbon bonds, resulting in a pair of aldehydes. The oxidation step must be regulated so as not to oxidise the aldehyde groups further.

The aldehyde groups are detected by Schiff's Reagent when exposed to it. The Schiff's Reagent reacts with the aldehyde groups, forming a colourless, unstable dialdehyde compound, which transforms to an insoluble magenta colored complex by restoration of quinoid chromophoric grouping.

REAGENTS PROVIDED

Kit Contents	Product Code	Storage Conditions	Pack Sizes		
			100ml	250ml	500ml
Diastase Solution (for PAS) (Reagent A)	SS004	2-8°C	100ml	250ml	500ml
Periodic Acid Solution - A (Reagent B)	IPS018	2-8°C	100ml	250ml	500ml
Schiff's Reagent (Reagent C)	SS003	2-8°C	100ml	250ml	500ml
Modified Mayer's Hematoxylin (Reagent D)	PS020	RT	100ml	250ml	500ml

STORAGE AND HANDLING

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

Laboratory Use Only

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

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

SPECIMEN PREPARATION

Recommended positive controls: Liver, Hepatocellular carcinoma

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

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; 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)
- Bluing solution
- DPX Mountant
- Microscopic slides (positively charged)
- Slide holder
- Jars
- Cover slips
- Coplin jars
- Drying oven

STAINING PROCEDURE:

1. Deparaffinize and rehydrate the tissue sections.
2. Preheat the Diastase Solution (for PAS) (Reagent A) at 37°C for 5-15 minutes.

Note: Do not overheat the solution above 40°C, as the enzyme activity can be degraded at higher temperatures. Insufficient heating may cause incomplete digestion of glycogen.

3. Treat the sections with warm Diastase Solution (for PAS) for 20-30 minutes at 37°C in an incubator or water bath.
4. Wash well in running tap water.
5. Incubate the sections using Periodic Acid Solution - A (Reagent B) for 5 minutes.
6. Rinse in distilled water for 1-2 minutes.
7. Cover the sections with Schiff's Reagent (Reagent C) in a dark staining chamber for 5-15 minutes.

Note: Schiff's Reagent is photosensitive and should be used and stored away from light.

8. Wash in running tap water for 5-10 minutes.
9. Counter stain with Modified Mayer's Hematoxylin (Reagent D) for approximately 15 seconds.
10. Wash in tap water.
11. Dehydrate using graded alcohol (70%, 80%, 95%, and 100%) for 2 minutes each.
12. Clear in xylenes and mount with DPX Mountant.

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Laboratory Use Only

QUALITY CONTROL

The recommended positive tissue control for the PAS-Diastase (PAD) Stain Kit is FFPE sections of Liver, Hepatocellular Carcinoma.

PERFORMANCE CHARACTERISTICS

PAS Diastase kit stains **Mucins** in **Magenta**, **Nucleus** in **Purple or Dark Blue,** and **Glycogen** is **digested and not stained.**

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. PathnSitu makes no warranties beyond those expressly stated in the product description.
15. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
16. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
17. For detailed information and specifications on individual components, please refer to the Product Material Safety Data Sheet (MSDS)

BIBLIOGRAPHY

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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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