PAS-Diastase (PAD) Stain

Laboratory Use Only CE

PRODUCT INFORMATION:

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 stain 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 specifically identified in certain tissue samples using the PAS staining procedure only if the glycogen (which is also PAS-positive) is digested with diastase and washed out. In cirrhosis, α_1-AT globules characteristically occur at the periphery of the nodules in multiple sizes within the hepatocyte gives a dark, reddish-purple 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 to 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 oxidizing agent which oxidizes compounds having free hydroxyl groups or amino/alkylamine groups. The tissue sections are first oxidized using periodic acid which oxides the vicinal bonds in these sugars, breaking the carbon-carbon bonds resulting in the pair of aldehydes. The oxidation step has to be regulated as to not further oxidize the aldehyde groups.

The aldehyde groups are detected by schiff's reagent when exposed to it. The Schiff's reagent reacts with the aldehyde groups forming colorless, unstable dialdehyde compound which transforms to 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 (Reagent A)	SS004	2-8°C	100ml	250ml	500ml
Periodic Acid Solution (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 2-8°C. 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 room temperature away from sunlight in an upright position.

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, 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)
- 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 (Reagent A) at 37°C for 5-15 minutes.

Note: Do not over heat 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 15-20 minutes at 37°C in an incubator or water bath.
4. Wash well in running tap water.
5. Incubate the sections using Periodic Acid (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.

QUALITY CONTROL

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

PERFORMANCE CHARACTERISTICS

Mucins stains Magenta, Nucleus stains Purple or Dark Blue and Glycogen is digested and not stained.

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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.

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EXPLANATION OF SYMBOLS

LOT- Lot number / Batch number

- Expiry

Storage limitation

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RT- Room Temperature

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