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Chemistry beyond the ordinary

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Periodic Acid Schiff's Stain Kit (PAS)

INTRODUCTION

PAS techniques are used to demonstrate polysaccharides, neutral mucosubstances and basement membranes primarily in tissue.The PAS reagent is also called Fuelgen Stain for the demonstration of DNA with a different protocol. Kidney is the most sensitive control; however, the demonstration of glycogen is best represented by using a section of liver containing glycogen. If performing a PAS reaction with diastase digestion, use two liver sections containing glycogen, one labeled "with diastase" and one labeled "without diastase" to use as control.

The principle of the PAS reaction is the conversion or loss of the quinoid structure and the masking of chromophores. This forms a colorless compound called leuco-fuchsin which can be changed to a bright rose color by washing in running water removing the sulphurous groups and restoring the quinoid groups. Excess Schiff reagent is removed by potassium metabisulfite rinses. Thus preventing a false positive by oxidation of any loosely adsorbed reagent in the tissue.

FIXATION

Fresh tissue specimen for paraffin embedding, should be fixed in 10% neutral buffered formalin. Methyl alcohol for blood smears. Sections should be cut at 4µ to 5µ.

DEPARAFFINIZE

Slides should be deparaffinized through xylene or xylene substitute to remove the paraffin from the section and descending grades of alcohol to distilled water just prior to staining.

STAINING PROCEDURE

Routine Procedure for Room Temperature

1. Place slides in 0.5% Periodic Acid for 5 minutes
2. Wash slides in 3 changes of distilled water.
3. Place slides in Schiff's Reagent for 15 minutes. The Schiff's Reagent should be brought to room temperature prior to staining. Clean staining dishes or Coplin jars are required. The glassware should be acid cleaned to avoid contamination.
4. Wash for 1 minute each in 2 changes of 0.55% Potassium Metabisulfite to remove excess Schiff reagent.
5. Wash in running tap water for 10 minutes to allow the color to develop.
6. Counterstain with Acidified Harris Hematoxylin for 30 seconds.
7. Wash the slides in running tap water to blue the hematoxylin.
8. Dehydrate through 95% alcohol, and 100% alcohol and clear with xylene.
9. Coverslip with Poly-Mount® or other synthetic resin.

STAINING PROCEDURE: MICROWAVE PROCEDURE

Steps 1 to 4 must be done under a hood and the microwave must be vented or under the hood. Larger amounts of stain can be made and heated by extending the times as indicated in the procedure. Heating in a Coplin jar will require 15 to 30 seconds to reach 60° C and 1 to 1 1/2 minutes for 250 ml to reach 60° C in a larger staining dish on HIGH in a microwave or as directed by the manufacturer using a probe. Please calibrate your oven by checking this temperature with room temperature distilled water for accurate control.

1. The glassware should be acid cleaned to avoid contamination.
2. Preheat the 0.5% Periodic Acid Solution in the microwave for 15 to 30 seconds for 50ml in a Coplin jar or 1 to 1 1/2 minutes for 250mL of solution in a large staining dish. Place slides in warm solution for 2 minutes.
3. Wash slides in 3 changes of distilled water.
4. Preheat the Schiff's reagent for 15 to 30 seconds with 50 ml in a Coplin jar or 1 to 1 1/2 minutes with 250 ml in a large staining dish prior to staining. Place the slides in the Schiff's reagent for 1 to 2 minutes.
5. Wash for 1 minute each in 2 changes of 0.55% Potassium Metabisulfite to remove excess Schiff reagent.
6. Wash in running tap water for 10 minutes to allow the color to develop.
7. Counterstain with Acidified Harris Hematoxylin for 30 seconds.
8. Wash the slides in running tap water to blue the hematoxylin.
9. Dehydrate through 95% alcohol, and 100% alcohol, clear with xylene.
10. Coverslip with Poly-Mount® or other synthetic resin.

PAS WITH DIASTASE DIGESTION

The digestion should be completed and the slides stained with the patient or test slides. The glycogen will not stain after digestion.

1. Use 100mg of Diastase powder, provided, to 50ml of pH 5.0 Phosphate Citrate Buffer. Stir until dissolved. Use immediately.
2. Place slides in the Diastase solution for 20 minutes. This solution can be warmed in the microwave however; it will change the pH and must be watched carefully to avoid over digestion of the tissue.
3. Wash in running tap water for 1 minute.
4. Stain as above for negative control.

RESULTS

Glycogen - Red Nuclei - Blue

Ordering Information:

Cat. #	Description	Size
24200-1	Periodic Acid Schiff's Stain Kit (PAS)	1 kit

Kit Contains:

- 250ml - 0.5% Periodic Acid Aq
- 250ml - Schiff' Reagent
- 250ml - 0.55% Potassium Metabisulfite
- 250ml - Harris Hematoxylin Acidified
- 250ml - Phosphate Citrate Buffer pH 5.0
- 0.5gm - Diastase Powder

Also Available:

08381-120	Poly-Mount®	120ml
08381-940	Poly-Mount®	940ml

TO ORDER

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