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## PAS Stain

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Works for me

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### ABSTRACT

This is a protocol for PAS Staining of paraffin embedded or cryo preserved tissue sections

### GUIDELINES

When preparing Coleman Schiff Reagent cover container of solution tightly when bleaching overnight

Test Coleman Schiff reagent prior to each use by adding a few drops to 5ml formaldehyde solution

Instant bright red/purple—good to use

Delayed blue/purple—discard

Make Periodic Acid Solution fresh each time

### MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
<a href="#">Hydrochloric acid</a>	<a href="#">H1758</a>	<a href="#">Sigma Aldrich</a>
<a href="#">Periodic acid</a>	<a href="#">P7875-25G</a>	<a href="#">Sigma Aldrich</a>

### MATERIALS TEXT

Basic Fuchsin Sigma Cat # 857343-25G

Potassium Disulfite Sigma Cat# P2522

Charcoal Sigma Cat# C5260

Xylene Fisher Cat# HC700

Ethanol PharmCo Cat# 11100020S

Harris Hematoxylin Sigma Cat# HHS32-1L

Ammonium Hydroxide Sigma Cat# 221228

### SAFETY WARNINGS

Wear PPE when working with Basic Fuchsin and while staining!

#### BEFORE STARTING

##### **0.5% Periodic Acid Solution**

\*Make fresh each time

Periodic Acid 1.5g

Distilled water 300mL

##### **1N Hydrochloric Acid Solution**

Hydrochloric Acid 83.5mL

Distilled water 916.5mL

##### **1% Ammonia**

Ammonium Hydroxide 10mL

Distilled water 990mL

### **Acid/Ethanol Solution**

Hydrochloric Acid 5mL

Ethanol 700mL

Distilled water 295mL

### **Preparation of Coleman's Schiff Reagent:**

- Heat 300ml distilled water to ~60C, just before boiling (10-15 min)
  - o Add 1.5g Basic Fuchsin
- Cool Solution (about 2 hr)
  - o Add 3.0g Potassium disulfite
  - o Add 15mL 1N HCl
- Let solution bleach overnight in the dark for 24hrs (solution will be yellowish/brown)
  - o Make sure solution is tightly closed (I wrap parafilm around the foil covered beaker)
- Add 1.5g Charcoal, stir for 1-5 minutes
  - o Filter through coarse filter paper
- Store at 4C, light protected

- 1 Deparaffinize and hydrate to water
- 2 Oxidize in periodic acid solution for 5 minutes
- 3 Rinse in distilled water
- 4 Place in Coleman's Schiff reagent for 15 minutes
- 5 Rinse in running tap water for 10 minutes
- 6 Counterstain in Harris Hematoxylin for 3 minutes
- 7 Wash in running tap water until water runs clear
- 8 10 dips in 0.5% HCl/70% Ethanol
- 9 Rinse in running tap water for 30 seconds
- 10 10 dips in 1% Ammonia water for 30 seconds
- 11 95% Ethanol, 2X, 2 minutes each

12 100% Ethanol, 2X, 2 minutes each

13 Xylene, 2X, 2 minutes each

14 Coverslip with Cytoseal



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