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PAS Stain

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Protocol status: Working

We use this protocol and it's working

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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

MATERIALS

 Hydrochloric acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #H1758**

 Periodic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7875-25G**

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

Troubleshooting

Safety warnings

 Wear PPE when working with Basic Fuchsin and while staining!



Before start

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