



KIDDRC
Histology Services
Protocols

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PERIODIC ACID-SCHIFF (PAS)

Periodic acid

.5% periodic acid in DHOH

Schiff's reagent

Dissolve 1g. basic fuchsin in 200ml of boiling distilled water; removing the flask of water from heat just before adding the basic fuchsin (do not add while still on hotplate). Allow to cool to 50°C, then add 2g. potassium metabisulphite with mixing. Allow to cool to room temperature then add 2 ml HCL, mix, add 2 g activated charcoal and leave overnight in the dark at room temperature. Filter through a #1 Whatman paper when the solution is either clear or pale yellow. Store in dark container at 4°C.

1. Dewax and bring to DHOH.
2. Treat with periodic acid for 5 minutes.
3. Wash well with several changes of DHOH.
4. Stain with Schiff's reagent for 15-20 minutes.
5. Wash in running tap water 5-10 minutes.
6. Stain nuclei with hematoxylin (3 dips for Viju's samples), differentiating with acid alcohol and blueing as usual.
7. Dehydrate to xylene substitute and coverslip.

Results:

glycogen and other periodic-reactive carbohydrates--magenta nuclei--blue

AMYLASE DIGESTION:

1% Amylase in DHOH

After dewaxing and hydrating slides, treat in amylase for 1 hour at 37°C. Rinse 5-10 minutes in running tap water; then continue with PAS stain.

From the internet:

Description: This method is used for detection of glycogen in tissues such as liver; cardiac and skeletal muscle on formalin-fixed, paraffin-embedded tissue sections, and may be used for frozen sections as well. The glycogen, mucin, and fungi will be stained purple and the nuclei will be stained blue.

Fixation: 10% formalin.

Section: paraffin sections at 5 μ m.

Solutions and Reagents:

0.5% Periodic Acid Solution:

Periodic acid ----- 0.5 g

Distilled water ----- 100 ml

Schiff Reagent:

Test for Schiff reagent: Pour 10 ml of 37% formalin into a watch glass. To this add a few drops of the Schiff reagent to be tested. A good Schiff reagent will rapidly turn a red-purple color. A deteriorating schiff reagent will give a delayed reaction and the color produced will be a deep blue-purple.

Mayer's Hematoxylin Solution:

Procedure:

- 1.** Deparaffinize and hydrate to water.
- 2.** Oxidize in 0.5% periodic acid solution for 5 minutes.
- 3.** Rinse in distilled water.
- 4.** Place in Schiff reagent for 15 minutes (Sections become light pink color during this step).
- 5.** Wash in lukewarm tap water for 5 minutes (Immediately sections turn dark pink color).
- 6.** Counterstain in Mayer's hematoxylin for 1 minute.
- 7.** Wash in tap water for 5 minutes.
- 8.** Dehydrate and coverslip using a synthetic mounting medium.