

PERIODIC ACID SCHIFF (PAS) PROTOCOL

PRINCIPLE:

This stain is used for the demonstration of glycogen. Tissue sections are first oxidized by periodic acid. The oxidative process results in the formation of aldehyde groupings through carbon-to-carbon bond cleavage. Free hydroxyl groups should be present for oxidation to take place. Oxidation is completed when it reaches the aldehyde stage. The aldehyde groups are detected by the Schiff reagent. A colorless, unstable dialdehyde compound is formed and then transformed to the colored final product by restoration of the quinoid chromophoric grouping.

QUALITY ASSURANCE:

The PAS stain with diastase or -amylase digestion has histochemical specificity for glycogen. Skeletal muscle normally contains glycogen and is often recommended as a positive control tissue.

SPECIMEN REQUIRED: Snap frozen human striated muscle. (Use the isopentane freezing method previously described.)

METHOD:

Fixation: None, use snap frozen tissue.

Technique: Cut 10 - 16 micron (12 μ m) sections in cryostat from snap frozen biopsy. Attach one or more sections to a No.1_, 22 mm square coverslip.

Equipment:

Ceramic staining rack - Thomas Scientific #8542-E40
Columbia staining dish - Thomas Scientific #8542-C12
Columbia staining dish (jar) - Thomas Scientific #8542-E30
Forceps
Latex gloves

Reagents:

- Absolute alcohol (100% ethanol) - Quantum, **FLAMMABLE** store at room temp. in a flammable cabinet
- Glacial Acetic Acid -Fisher A507-500, **CORROSIVE** store at room temperature
- Amylase - Sigma A-6505, store at room temperature
- Chloroform - Baxter 049-4, **FLAMMABLE CARCINOGEN** store at room temperature in a flammable cabinet)
- Periodic Acid - Sigma P7875, store at room temperature
- Permout - Fisher SP15-100, **FLAMMABLE HEALTH HAZARD**
- Reagent alcohol, ACS - histological Fisher A962-4 or HPLC A995, **FLAMMABLE, TOXIC, TERATOGENIC**, store at room temperature In flammable cabinet
- Schiff Reagent - Harleco 6073/71, store at room temperature
- Xylenes - Fisher #HC700-1GAL, **FLAMMABLE**, store room temperature in flammable cabinet)

Solutions:

I. Carnoy's Fixative (store at room temperature) **PREPARE IN A FUME HOOD**

Alcohol, 100 % 60 ml

Chloroform 30 ml

Glacial acetic acid 10 ml

2. Periodic Acid Solution, 0.5 % (w/v) **PREPARE FRESH FOR EACH STAIN**

Periodic acid 50 mg dissolved in deionized water 10 ml

3. Alcohol 50 %

Reagent alcohol ~50 ml

Deionized water ~50 ml

4. Alcohol 70 %

Reagent alcohol ~70 ml

Deionized water ~30 ml

5. Alcohol 80 %

Reagent alcohol ~80 ml

Deionized water ~20 ml

6. Alcohol 95 %

Reagent alcohol ~95 ml

Deionized water ~ 5 ml

Staining Procedure:

1. Place the coverslip with section in a columbia staining dish (Thomas Scientific #8542- E40).
2. Add Carnoy's fixative to dish for 10 minutes.
3. **Rinse very carefully with several exchanges of deionized water. Sections may wash off!!**
4. Add Periodic Acid solution to staining dish for 10 minutes.
5. **Rinse very carefully with several exchanges of deionized water. Sections may wash off!!**
6. Add Shiff Reagent for 5 minutes
7. Carefully wash with three exchanges of tap or deionized H₂O.
8. Dehydrate in ascending alcohol solutions (50%, 70%, 80%, 95% x 2, 100% x 2) in columbia staining dish(jar)s - Thomas Scientific #8542-E30.
9. Clear with xylene (3 - 4 x) also in columbia staining dish(jar) - Thomas Scientific #8542-E30.
10. Mount coverslip onto a labeled glass slide with Permount or some other suitable organic mounting medium

Results:

Glycogen, neutral mucosubstances, basement membranes, collagen fibers, glycolipids and phospholipids will be demonstrated as pink to red to purple color.

If diastase or -amylase is used for a negative control, the glycogen deposits are removed leaving only the plasma membrane staining pink. The two major types of fibers are usually distinguished by different intensity of staining.

REFERENCES:

1. Thompson, Samuel W. *SELECTED HISTOCHEMICAL AND HISTOPATHOLOGICAL METHODS*, Charles C. Thomas, Springfield, IL, 1966.
2. Sheehan, D.C. and Hrapchak, B.B., *THEORY AND PRACTICE OF HISTOTECHNOLOGY*, 2nd Edition; Battelle Memorial Institute, Columbus, OH, 1987.