PAS Staining of Fresh Frozen or Paraffin Embedded Human Kidney Tissue V.2

Elizabeth Neumann[1], Jamie Allen[1], Jennifer Harvey[1], Maya Brewer[2], Carrie Romer[1], Mark De Caestecker[2], Jeff Spraggins[1]

[1]Vanderbilt University; [2]Division of Nephrology, Vanderbilt University Medical Center

Version 2

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1 Works for me

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VU Biomolecular Multimodal Imaging Center
Tech. support email: jeff.spraggins@vanderbilte.du

Jamie Allen
Vanderbilt University

ABSTRACT

Scope:
The PAS stain is used to demonstrate polysaccharides such as glycogen, and mucosubstances such as glycoproteins, glycolipids and mucins in tissues. It is used as a replacement for the H&E in kidney pathology.

Expected Outcome:
Intermyofibrillar Network.....Pink to Rose
Glycogen.....Pink to Rose
Myofibrils.....Unstained
Type I fibers.....Lighter
Type II fibers.....Darker
Blood Vessel walls & connective tissue.....Faintly Stained

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

References: 1. Luna, Lee (ed.). Manual of histological staining methods of the Armed Forces Institute of Pathology. 2. Dubowitz, B. Muscle Biopsy. A practical approach, 2nd edition, Bailliere, Tindall, London, 1985. 3. Dr. Fogo Clinical lab PAS protocol.

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GUIDELINES

1. Leftover matrix will change the pH of the solutions and prevent adequate staining.

2. Bubbles left on tissue will prevent acquisition of high-quality microscopy images.

MATERIALS TEXT

Reagents:
1. Commercial kit AbCam 150680
2. Ethanol, Fisher BP2818500
3. Hematoxylin Solution, Mayer's, Sigma MHS32-1L
4. Xylenes, Histological Grade, Sigma 534056

Materials:
1. Easy Dip Slide Staining Jars, Mercedes Medical SIM M90012AS
2. Coplin Dish Staining Dish, Fisher S17495
3. Microscope Cover Slips, Creative Waste Solutions

Solutions:
1. 0.5% Periodic Acid:
   Periodic Acid……5g
   Distilled Water….1000mL
   Pour out what is needed and reuse for ~1 month
   Store at room temperature for 6 months
2. Schiff's Reagent – Commercially Prepared
   Richard Allan Scientific Catalog number: 88017
   Solution may be reused for ~1 month.
   ***Check for effectiveness by placing 3 drops of formalin in 2mL Schiff's reagent.
   The solution should immediately turn purple. Follow manufacturer expiration date on bottle.
3. Stock of 0.5% Ammonium Hydroxide
   2.5mL of Ammonium Hydroxide
   497.5mL Milli-Q H_2O
4. Hematoxylin (filtered at least 1x/week)
5. Ethanol solutions
   70% - 350mL EtOH + 150mL Milli-Q H_2O
   95% - 350mL EtOH + 25mL Milli-Q H_2O

SAFETY WARNINGS

1. Safety glasses or goggles, proper gloves, and a lab coat required. The area should be adequately vented and a lab mat placed underneath all solutions.

2. Xylenes should be used in the fume hood.

Start with FFPE here:

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1 Allow PAS "kit" to come to room temperature on the bench.

2 For paraffin sections, deparaffinize in xylene, two changes, **00:03:00** each.

3 Hydrate through graded alcohols, **00:01:00** each:
100%, 100%, 95%, 70%, water

4 Rinse well in distilled water by holding finger over slides, pouring water into sink and adding water. Do this 5 times.

Start Frozen samples here:

5 Remove frozen slides from freezer and let equilibrate to room temperature, and then place in 10% Formalin for **05:00:00** . Proceed to step 7.

6 If staining is performed following MALDI analysis and samples have matrix on them, remove matrix in 90% ethanol (~2-3 min or until matrix is gone) **00:03:00 Until Matrix is removed** then in 70% ethanol for **00:03:00** . Proceed to step 7.

7 Rinse with distilled water (follow Step 4).

8 Place in 0.5% Periodic acid for **00:10:00** (to oxidize).

9 Rinse well in distilled water (follow Step 4).

10 Pour Schiff's reagent into Coplin jar containing slides. Allow to sit 15-30 minutes at room temperature (kidney samples ~30 minutes).

11 Rinse in running warm (~40 °C) distilled or tap water (follow Step 4).

The tissue should appear pink after rinsing with warm water.

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12 Place slides in movable gray slide holder.

13 Counterstain in Hematoxylin (staining line) for **00:01:00** .

14 Rinse well in distilled water, starting with blue container next to hematoxylin (follow Step 4).

15 Quickly dip slides into “Bluing” agent (0.5% ammonium hydroxide).

16 Rinse 1 minute in distilled water until pink color is visible (follow Step 4).

17 Dehydrate through graded alcohols, 10 short dips in each:
95%, 95%, 100%, 100%

If the last 100% ethanol rinse is colored by residual stain, additional washing in new 100% ethanol solution should be performed.

18 Fix in 2 rounds of xylenes, **00:01:00** each (in the hood).

19 Coverslip slides:
1. Place coverslip on paper towel
2. Add 2-3 drops of cytoseal to edge (depending on size)
3. Dip slide into xylenes, take out and roll the xylene lengthwise on slide
4. Line up slide and cover slip and slowly place the slide on the coverslip
5. If needed, use dissecting tool to remove bubbles

20 Image slides using a Leica brightfield scanner (or equivalent) at 20x (~1 µm spatial resolution) and save as .tiff or .jpg file.

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