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 We use this protocol and it's working

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PAS Staining of Fresh Frozen or Paraffin Embedded Human Kidney Tissue V.1

Jamie Allen¹, Jennifer Harvey¹, Maya Brewer², Mark De Caestecker², Jeff Spraggins¹

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

VU Biomolecular Multimodal Imaging Center / Spraggins Research Group

Human BioMolecular Atlas Program (HuBMAP) Method Development

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

MATERIALS

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 discard
 Store at room temperature for 6 months
2. Schiff's Reagent – Commercially Prepared
 Richard Allan Scientific Catalog number: 88017
 Solution may be reused several times.
 ***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 H₂O
4. Hematoxylin (filtered at least 1x/week)
5. Ethanol solutions
 70% - 350mL EtOH + 150mL Milli-Q H₂O
 95% - 350mL EtOH + 25mL Milli-Q H₂O

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:

- 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 straining 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 00:01:00 . Proceed to step 7.
- 7 Rinse with faucet water (follow Step 4)
- 8 Place in 0.5% Periodic acid for 00:10:00 10 minutes (to oxidize)
- 9 Rinse well in distilled water (follow Step 4)
- 10 Pour Schiff's reagent into copland jar containing slides. Allow to sit 15-30 minutes at room temperature (kidney samples ~30 minutes)
- 11 Rinse in running warm tap water (follow Step 4)
- 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%

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

19 Coverslip slides:
1. Place coverslip on dry towel
2. Add 2-3 drops of cyto seal 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