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U Michigan - Mesangial matrix evalutaion V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.562g9ge

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ABSTRACT

Summary:

Periodic acid-Schiff (PAS) is a staining method used to detect glycogen on formalin-fixed, paraffin-embedded kidney tissue sections. PAS staining highlights basement membranes and is frequently used to diagnose glomerular mesangial matrix expansion.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=312&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Staining Jars	22038493	Fisher Scientific
Periodic acid	P7875-25G	Sigma Aldrich
Schiff's reagent	3952016-500ML	Sigma Aldrich
Gill 2 Hematoxylin	72511	Richard-Allan Scientifi
Xylene	X3P-1GAL	Fisher Scientific
Ethanol (EtOH) 200 Proof	2701	Decon-Laboratories Inc
Tissue sections		
Mounting Medium	8312-4	Thermo Scientific
Cover Glass	12-542-B	Fisher Scientific
Universal Imaging MetaMorph® Imaging System		Molecular Devices
Scientific grade digital color CCD camera		RT SLIDER DIAGNOSTIC
Microscope and Lense		Leica DM IRB

MATERIALS TEXT

Note:

Thermo Fisher Scientific, RRID:SCR_008452
Sigma-Aldrich, RRID:SCR_008988
Leica Microsystems, RRID:SCR_008960

Protocol 1: PERIODIC ACID SCHIFF (PAS) STAINING

1 1. Wash 2x 4 minutes in Xylene.
2. Wash in 100% E_tOH 2x minutes
3. Wash in 95% E_tOH 1x 2minutes.
4. Wash in 70% E_tOH 1x 2minutes
5. Rinse in dH_2O.
6. Incubate in 0.5% Periodic Acid solution for 5min.
7. Rinse 3x in dH_2O.
8. Incubate in Schiff's reagent for 15minutes
9. Rinse under running lukewarm tap water for 5minutes.
10. Incubate in Hemotoxylin for 90 second.
11. Rinse 6x in dH_2O.
12. Wash in 70% E_tOH 1x 2minutes.
13. Wash in 95% E_tOH 1x 2minutes.
14. Wash in 100% E_tOH 2x 2minutes.
15. Incubate in Xylene for at least 5minutes.
16. Mount slides with mounting medium 1 drop
17. Insert cover glass carefully, avoid bubble

Note: Schiff is light sensitive, mutagenic and has bad smell. *Use fume hood or flow hood for handling* Schiff's reagent.

Protocol 2: MESANGIAL MATRIX QUANTIFICATION

2 **Pre-Operating Instructions:**
Camera and Microscope should be calibrated and values loaded into MetaMorph® Program

1. Using the camera or MetaMorph® software digitizes 30 cortical glomeruli per case with a 40 X lens. Glomeruli should be chosen for a similar diameter of maximal size. Save images as uncompressed Tiff files.

2. Open the glomerulus Tiff file in MetaMorph®. Scale the image in such a way that the entire glomerulus can be seen on the screen (50-75%).

3. Using the polygon tool carefully outline the glomerular tuft. Double click to close the polygon tool.

4. From the tool bar choose **Measure, Calibrate Distances** and in the **Apply** window choose the calibration file for the camera from which the image was taken. Then choose **Apply.**

5. The area of the glomerular tuft can then be calculated by choosing **Measure** from the tool bar and then **Region Measurements**. Record the glomerulus area displayed.

6. To calculate the area that is PAS stained the tuft will have to be removed from the background. Select the outlined tuft and then from the tool bar choose **Edit, Duplicate, Image.** Close the full size image.

7. Size the edited image to 150 – 200 %.

8. From the tool bar select **Measure** and **Set Color Threshold**.

9. In the **Set Color Threshold** window select **Set By Example**.

10. Using **Display** on the tool bar and **Adjust Digital Contrast** the color brightness and contrast can be adjusted to best suit thresholding the area of PAS stain.

11. Once the image is adjusted use the cursor to choose the area of staining. Continue clicking the cursor over the area until the entire **PAS** stained area is highlighted. As each pixel is selected every pixel that color is also selected. Care must be taken to ensure that ***only*** **PAS** stained tissue is highlighted.

♦ **NOTE:** If an area is mistakenly selected selecting **Undo Last Click** in the **Set Color Threshold** box will remove the last selection.

♦ To clear all selections check the box next to **Reset color threshold range on next click** in the **Set Color Threshold** box. The next pixel chosen in the image will clear the screen. Continue selecting pixels.

♦ To toggle between the selected and unselected screen, select **OFF** and **Inclusive** in the **Set Color Threshold** box

12. Once all the PAS stained area is selected choose **Measure** and **Integrated Morphometry Analysis**.

13. In the **Integrated Morphometry Analysis** box under **Set Up Parameters** For: choose **Measuring** and then **Total Area** and then Classifying and Total Area.

14. Under **Display** choose **Summary**.

15. Under **Show/Log Data** choose **Current**.

16. Now choose **Measure** and the "area of PAS staining" will be the last cell in the **Summary** window under **Total**.

17. To close the **Integrated Morphometry Analysis** box choose **Reset Current** and then **Close**.

18. The percent of the glomerulus that is PAS stained is calculated as:

$$\left(\frac{\text{Area of PAS staining}}{\text{Total area of glomerulus}} \right) \text{X } 100$$