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WT-1 Staining Protocol for Podocytes

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1 Works for me dx.doi.org/10.17504/protocols.io.36ggrbw

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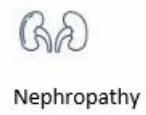
ABSTRACT

Summary:

This protocol describes a protocol used by some DiaComp members to detect podocyte nuclei in rodent glomeruli.

Edited by: Brosius Laboratory

Diabetic Complications:



EXTERNAL LINK

https://diacomp.org/shared/document.aspx?id=63&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
75% 90% and 100% Ethanol		
PBS pH 7.4		
Hydrogen peroxide		
Retrieve-All 1(1x)	1912	Signet Pathology Systems
1% BSA in PBS		
WT1(C-19) rabbit polyclonal IgG	sc-192	Santa Cruz Biotechnology
DAB (Diaminobenzidine) Tablets	D-4293	Sigma Aldrich
Methanol		
Pemount		
Xylene		
Coverslips		
Staining Dish		

NAME	CATALOG #	VENDOR
Staining Rack		
Staining Dish for boiling slides	25608-904	VWR Scientific
Vectastain ABC Kit Rabbit IgG	PK-6101	Vector Laboratories

MATERIALS TEXT

Reagent/Material	Quantity Required
Vectastain ABC Kit, Rabbit IgG	1
Retrieve-All 1(1x)	1
Staining Dish	12
Staining Rack	2

Note:

Santa Cruz Biotechnology (RRID:SCR_008987)

Sigma-Aldrich (RRID:SCR_008988)

Vector Laboratories Cat# PK-6101, RRID:AB_2336820

1 Day 1. Deparaffinize/hydrate

1. Dip in Xylene 5 minutes 2x (carry out in the hood)
2. Dip in 100% ETOH 5 minutes 2X
3. Dip in 95% ETOH 5 minutes once
4. Dip in 70% ETOH 5 minutes in once
5. Dip in dH_20 5 minutes 2X
6. Dip in PBS 5 minutes once
7. Place the slides in jar with preheated Retrieve All 1 and incubate 2 hrs in 90°C water bath
8. Remove and let it cool down for 5-10 minutes
9. Pour off solution (can be reused 5-10 times) and add dH_2O
10. Rinse slides in two change of dH_2O 4-5 minutes each
11. Soak in PBS and store at 4°C.

2 Day 2. Immunoperoxidase

1. Blot dry slide (leave the section wet)
2. Incubate 20 minutes at room temperature (RT) with blocking serum (Follow directions of Vectastain ABS Kit for dilution).

3. Dilute WT-1 antibody (first antibody): 1:200 dilution in 1%BSA. Blot excess serum and incubate with first antibody for 2 hours at RT (2ug/section in 100ul).

4. Wash in PBS 5-10 minutes (2x changes) and blot dry (don't touch the section)

5. Incubate with second antibody for 1 hour (Follow directions of Vectastain ABS Kit for dilution)

6. Wash in PBS 5-10 minutes (2x changes)

7. Quench endogenous peroxidase by dipping in 1% H_2O_2 inmethanol for 45 minutes

8. Wash in PBS 10-15 minutes (2x changes)

9. Incubate with ABC Reagent for 1 hour (Follow direction ofVectastain ABS Kit for dilution).

10. Wash in PBS 5-10 minutes and prepare DAB Tablets (dissolve 1 set in 5 ml ddH_2O and filter it)

11. Staining: add 100ul or so DAB substrate to cover the whole section and check under the microscope for podocyte nuclei (~1 minute).

12. Stop reaction in dH_2O

13. Dip in dH_2O for 5 minutes 2x

14. Dip in 70% ETOH 5 minutes once

15. Dip in 95% ETOH 5 minutes once

16. Dip in 100% ETOH 5 minutes 2X

17. Dip in Xylene 5 minutes 2x (carry out in the hood)

18. Mount the slides with permount.