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Version 2

WT-1 Staining Protocol for Podocytes V.2

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Protocol status: Working

We use this protocol and it's working

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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:



Nephropathy

Materials

MATERIALS

75% 90% and 100% Ethanol

PBS pH 7.4

Hydrogen peroxide

Retrieve-All 1(1x) **Signet Pathology Systems Catalog #1912**

1% BSA in PBS

WT1(C-19) rabbit polyclonal IgG **Santa Cruz Biotechnology Catalog #sc-192**

DAB (Diaminobenzidine) Tablets **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D-4293**

Methanol

Pemount

Xylene

Coverslips

Staining Dish

Staining Rack

Staining Dish for boiling slides **VWR International (Avantor) Catalog #25608-904**

Vectastain ABC Kit Rabbit IgG **Vector Laboratories Catalog #PK-6101**

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 dH₂O 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 dH₂O
10. Rinse slides in two change of dH₂O 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% H₂O₂ in methanol for 45 minutes
8. Wash in PBS 10-15 minutes (2x changes)
9. Incubate with ABC Reagent for 1 hour (Follow direction of Vectastain ABS Kit for dilution).
10. Wash in PBS 5-10 minutes and prepare DAB Tablets (dissolve 1 set in 5 ml ddH₂O 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 dH₂O
13. Dip in dH₂O 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.