



# WT-1 Staining Protocol for podocytes

Version: 1.0

Replaced by version: N/A

Edited by: Brosius Laboratory

Summary

Reagents and Materials

Protocol

**Summary:** This protocol describes a protocol used by some AMDCC members to detect podocyte nuclei in rodent glomeruli.

## Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
Vectastain ABC Kit, Rabbit IgG	1	Vector Laboratories Inc	PK-6101
75%, 90% and 100% Ethanol			
PBS, pH 7.4			
Hydrogen peroxide			
Retrieve-All 1(1x)	1	Signet Pathology Systems	1912
1% BSA in PBS			
WT1(C-19) rabbit polyclonal IgG		Santa Cruz	sc-192
DAB (Diaminobenzidine) Tablets		Sigma	D-4293
Methanol			
Pemount			
Xylene			
Coverslips			
Staining Dish	12		
Staining Rack	2		
Staining Dish for boiling slides		VWR	25608-904

### Protocol:

#### 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<sub>2</sub>O 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<sub>2</sub>O
10. Rinse slides in two change of dH<sub>2</sub>O 4-5 minutes each
11. Soak in PBS and store at 4<sup>0</sup>C.

**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<sub>2</sub>O<sub>2</sub> 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 ddH<sub>2</sub>O 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<sub>2</sub>O
13. Dip in dH<sub>2</sub>O 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.