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WT-1 Staining Protocol for Podocytes V.2 [↗](#)Frank Brosius<sup>1</sup><sup>1</sup>University of Arizona

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Works for me

[dx.doi.org/10.17504/protocols.io.8e2htge](https://dx.doi.org/10.17504/protocols.io.8e2htge)

Diabetic Complications Consortium

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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

## 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](#)

| NAME ▾                           | CATALOG # ▾ | VENDOR ▾            |
|----------------------------------|-------------|---------------------|
| Staining Dish                    |             |                     |
| 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<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°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<sub>2</sub>O<sub>2</sub> 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<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.



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