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# U Michigan - Intra-Epidermal Fiber Density V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.563g9gn

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ABSTRACT

**Summary:**

Intra-epidermal nerve fiber density (IENFD) is used as a tool to assess small fiber neuropathy.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=319&docType=Protocol

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Zamboni fix | # 1459A | Newcomer Supply |
| cryomold | Cryomold | tissuetek |
| sucrose | sucrose | Fisher Scientific |
| PGP 9.5 | 14730-1-ap | Proteintech |
| BSA | | Sigma Aldrich |
| Coverslip 1.5 | | Fisher Scientific |
| slides | | Fisher Scientific |
| Prolong gold w/ dapi | P36931 | Thermofisher |
| Secondary 488 g anti mouse highly cross absorbed | A31620 | Invitrogen - Thermo Fisher |

MATERIALS TEXT

**Reagent Preparation:**

Reagent 1:
**Blocking Solution**
Reagents and Materials
1) 5% BSA made in .3% triton tx100 in .1m PBS 7.4 pH

Reagent 2:
**Primary antibody**
Reagents and Materials
2) PGP antibody at 1/2000 made in 1% BSA in .3% triton tx100 in .1m PBS 7.4 pH

Reagent 3:
**Secondary**
Reagents and Materials
3) Molecular Probes anti-rabbit 488 highly cross absorbed secondary made in .3%

triton tx 100 in .1M PBS 7.4 pH.

**Note:**

**Thermo Fisher Scientific, RRID:SCR_008452**
**Sigma-Aldrich RRID:SCR_008988**
**Proteintech Group, RRID:SCR_008986**
**PGP9.5/UCHL1 Antibody,** Cite this **(Proteintech Group Cat# 14730-1-AP, RRID:AB_2210497)**

1 **Reagents:** Phosphate buffer (PB, 0.1 M, pH 7.2) Sucrose

**Equipment:** Razor blades, embedding molds, 24 well plate

**Solutions:** 2% zamboni's in phosphate buffer (PB, 0.1 M, pH 7.4)
30% sucrose in PBS

**Procedure:**

**Fresh mouse or rat:**
1. Remove footpad and fix (4 hours) in 2% zamboni's fixative for 4-6 hours.

2. Place skin in 30% sucrose in 1x PBS overnight.

3. Embed tissue in OCT using scheme below.

4. Cut 10-14 sections at 30um using scheme below and place into a single well of a 24 well plate.

5. Follow IHC protocol using Ptroteintech rabbit anti-PGP9.5(14730-1-ap), 1:2000 followed by AlexaFluor 488 highly cross absorbed, 1:1000 (Molecular Probes). Apply coverslips with ProLong gold or platinum antifade kit with dapi (Molecular Probes)

6. Block for 1-2 hours in blocking solution.

7, Primary at RT for 1 hour, then in to cold room overnight.

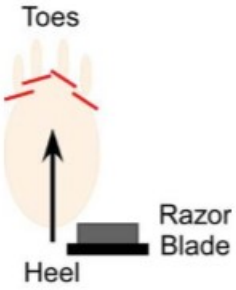
8. Rinse 3x1 hour in PBS

9. Secondaty at RT for 1 hour, then in to cold room overnight.

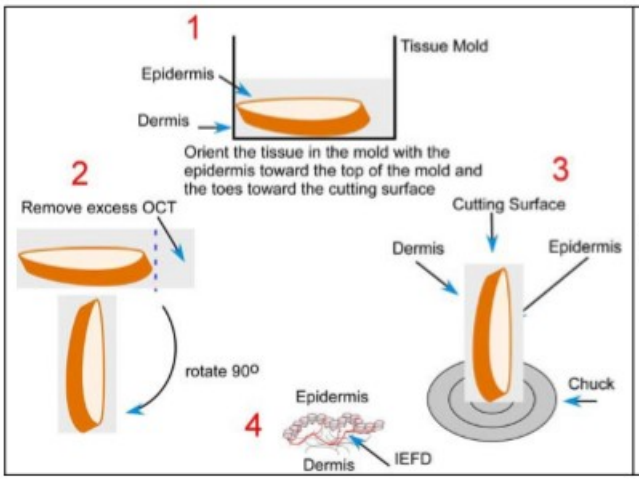
10. Rinse 3x1 hour in PBS.

11. Coverslip using dapi gold with prolong.

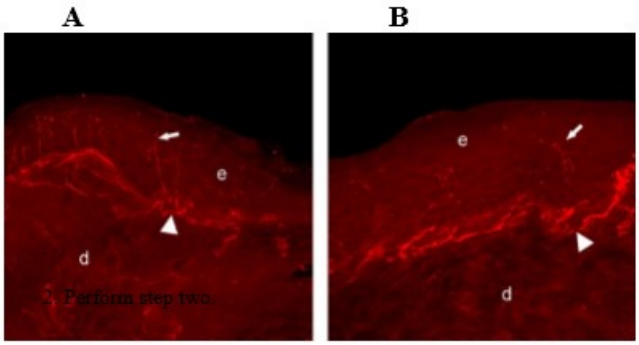
**Imaging:**
1. Three images per sample are collected on an Olympus FluoView 500 confocal microscope using a 20 X air objective at a resolution of 1024 X 1024 pixels. The optical section thicknessis3.3um. 10images per stack are flattened using MetaMorph(version7.70) arithmetic max option. Data are presented as the fibers crossing the epidermal border of PGP9.5 positive fibers per area of epidermis.




To dissect the foot pad, cut the skin at the toe joints with a sharp blade. Begin at the heel and move forward to remove the entire plantar surface of the foot. Handle the tissue by the heel only so as not to disrupt structures within the dermis and epidermis.




Embedding schema for all tissues in general and foot pads in particular.




IEFD in a control (A) and diabetic
(B) mouse