INTRA-EPIDERMAL FIBER DENSITY DETERMINATION OF RODENT FOOT PAD BIOPSIES

Version: 1
Replaced by version:
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(note that the following list should be linked to the appropriate location.)

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

Reagents and Materials: *(This should be a comprehensive list of stock solutions and material. The reagent list for the stock solutions is included in the reagent preparation area that is included at the end of this SOP.)*

Reagent/Material	Vendor	Stock Number
Zamboni fix	Newcomer Supply	# 1459A
cryomold	tissuetek	
sucrose	fisher	
PGP 9.5	Proteintech	14730-1-ap
BSA	sigma	
Coverslip 1.5	fisher	
slides	Fisher	
Prolong gold w/ dapi	thermofisher	P36931
Secondary 488 g anti	Invitrogen	A31620

mouse highly cross absorbed		

Protocol:

WARNING HAZARDOUS CONDITION WARNED AGAINST. This comment describes a hazardous condition to which the technician may be exposed in the performance of this protocol. It also contains directions on how to avoid or minimize the danger. Warnings are always and only used for personnel safety, and precedes the first step that will expose the technician to the hazard.

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:

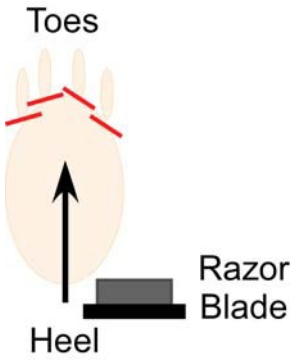
Fresh mouse or rat:

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.

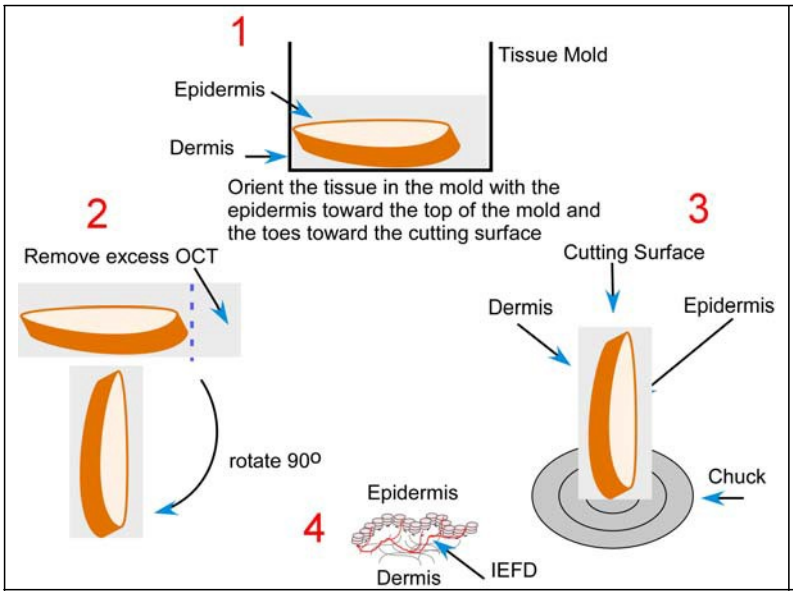
Imaging:

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 thickness is 3.3 um. 10 images per stack are flattened using MetaMorph (version 7.70) arithmetic max option. Data are presented as the fibers crossing the epidermal border of PGP9.5 positive fibers per area of epidermis.

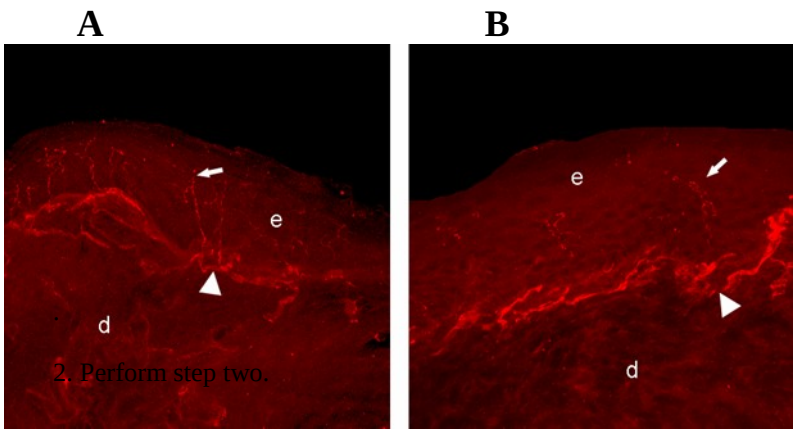
Figure 3



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

2. Perform step two.

Reagent Preparation: *(This area may have several different preparations with the table of contents below.)*

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.