Sep 27, 2019 Version 2

Neuropathy Phentoyping Protocols - Intra-Epidermal Fiber Density Determination of Rodent Foot Pad Biopsies V.2

DOI
dx.doi.org/10.17504/protocols.io.7rehm3e

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.7rehm3e

External link: https://www.diacomp.org/shared/document.aspx?id=54&docType=Protocol

Protocol Citation: Eva Feldman 2019. Neuropathy Phentoyping Protocols - Intra-Epidermal Fiber Density Determination of Rodent Foot Pad Biopsies. **protocols.io https://dx.doi.org/10.17504/protocols.io.7rehm3e**

Protocol status: Working
We use this protocol and it's working

Created: September 27, 2019

Last Modified: September 27, 2019

Protocol Integer ID: 28166

Keywords: Neuropathy Phentoyping, Diabetic Neuropathy, Intra-Epidermal Fiber Density, diabetic neuropathy at the whole animal, presence of diabetic neuropathy, development of diabetic neuropathy, diabetic neuropathy, detailed measures of neuropathy, assessments of neuropathy, neuropathy phentoyping protocol, neuropathy, rodent foot pad biopsies summary, diabetes, loss of nerve fiber, initial degree of neuropathy, duration of diabetes, diabetic complication, nerve fiber, degree of diabetes, month period of diabetes, tail blood glucose, degree of glycemic control, glycemic control, glycated hemoglobin level

Abstract

Summary:

Phenotyping of Rodents for the Presence of Diabetic Neuropathy
In man, the development of diabetic neuropathy is dependent on both the degree of glycemic control and the duration of diabetes. Diabetic neuropathy is a progressive disorder, with signs and symptoms that parallel the loss of nerve fibers over time. Consequently, assessments of neuropathy in mice are not performed at one time point, but are characterized at multiple time points during a 6 month period of diabetes. The degree of diabetes is evaluated in 2 ways: tail blood glucose measured following a 6 hour fast and glycated hemoglobin levels. The initial degree of neuropathy is screened using the methods discussed below. Detailed measures of neuropathy are employed when the initial screening instruments indicate a profound or unique phenotypic difference. This document contains protocols used by the DiaComp staff to examine and measure diabetic neuropathy at the whole animal, tissue and cellular levels.

Diabetic Complication:

Neuropathy

Materials

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

Equipment: Razor blades

Solutions: 4% paraformaldehyde in phosphate buffer (PB, 0.1 M, pH 7.2)
5, 10, and 20% sucrose in PB

Troubleshooting

1 **Perfused mouse or rat:**

1. Following perfusion, remove entire foot pad with a sharp razor blade. Begin by cutting skin between the plantar surface of the foot and the toes, then start at the heel to gently remove the skin and superficial fascia of the foot. Be careful to only handle the tissue by the heel.

2. Post-fix overnight in 4% paraformaldehyde.

3. Rinse in graded sucrose 5 – 20%, minimum 12 hours in each

2 **Fresh mouse or rat:**

1. Remove foot and fix overnight (12-18 hours) in 4% paraformaldehyde

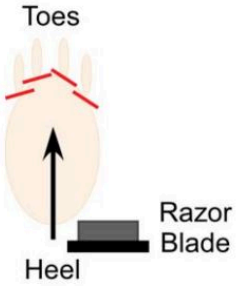
2. Remove skin and process as described above.

3. Follow IHC protocol using rabbit anti-PGP9.5, 1:1000 followed by AlexaFluor 598, 1:200 (Molecular Probes). Apply coverslips with ProLong antifade kit (Molecular Probes)

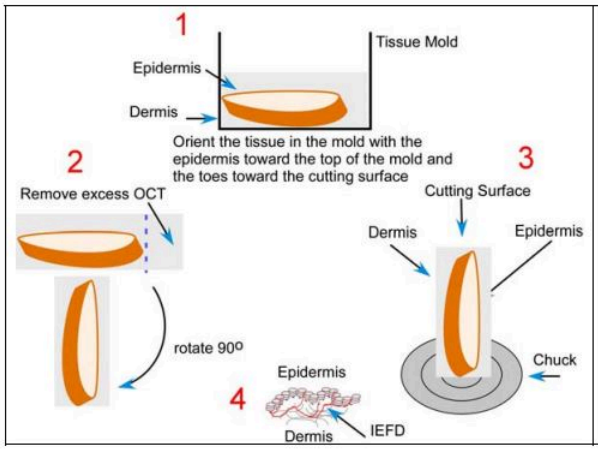
3 **Imaging:**

Three images per sample are collected on an Olympus FluoView 500 confocal microscope using a 60 X 1.2 water immersion objective at a resolution of 800 X 600 pixels. The optical section thickness is 0.5 um. Forty images per stack are flattened using MetaMorph (version 6.14) arithmetic option. Integrated morphometry analysis is used to exclude extraneous signals. The data are presented as the percent area of PGP9.5 positive fibers per area of epidermis.

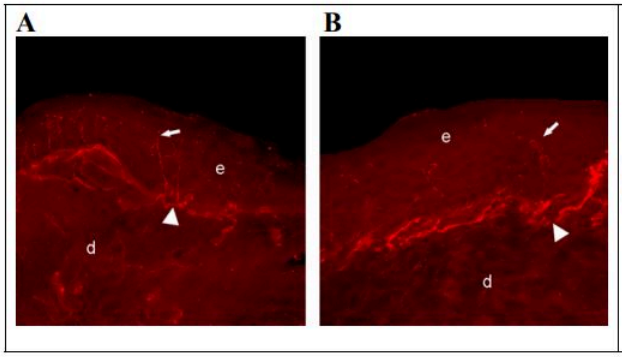
Figure 3



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