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# Neuropathy Phentoyping Protocols - Cryoembedding

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

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## 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

**Supplies:**
- Ice bucket
- Dry Ice (stored in –20ºC walk-in freezer)
- 2-methyl butane (stored in acid cabinet)
- Aluminum foil
- Small glass vial
- Weigh boats
- Forceps
- OCT (Optimal Cutting Temperature, Electron Microscopy Sciences)
- Alcohol-resistant marker (very important)

1 **Procedure: Fixed Tissue**

1. Following fixation (immersion or perfusion), rinse tissue in phosphate buffer (PB, 0.1M, pH 7.3) containing 5, 10, and 20% sucrose for 24 hours per step or until the tissue "sinks" to the bottom of the container or tube.

The final sucrose concentration in the tissue may vary from 5-30% depending on what conditions work best for the tissue in question.

2. Cool 2 methyl-butane in a dry ice bath until dry ice no longer boils when added.

3. Make embedding molds out of aluminum foil. Cut small squares, about 1" square or less. Use the bottom of a small glass vial or appropriately sized mold, form foil squares around bottom.

4. While foil is still on the mold, write all information concerning the tissue going in the mold.

5. Remove foil from mold, add OCT.

6. Take tissue out of 20% sucrose and place in a small dish or weigh boat containing OCT to remove any excess sucrose.

7. Place tissue in foil mold. Make sure all tissue is flat on the bottom of the mold. Cover tissue with OCT.

8. Using forceps, hold mold in 2-methyl butane until OCT freezes. Try not to drop the mold in the 2-methyl butane, as the block will freeze too fast and crack.

9. Keep in bucket on dry ice until all blocks are ready to be put in –20ºC or –80ºC freezer. Fold excess foil over the top of the block to prevent freeze-drying.

2 **Procedure: Fresh Tissue**

Blocks of fresh tissue may be snap frozen at approximately -40ºC and immediately sectioned on the cryostat or covered in OCT, snap frozen and stored at for later sectioning. The freezing temperature is critical, the slower the tissue freezes, the more ice crystals form. If the piece of tissue is very large there is a risk of it shattering if frozen too fast.