

# **SOP-NOD-0011 Mouse Necropsy**

## **T1D Mouse Necropsy for Pancreas and Related Organs**

### **T1D MOUSE NECROPSY FOR PANCREAS AND RELATED ORGANS**

#### **1. PURPOSE**

The purpose of this Standard Operating Procedure (SOP) is to outline proper procedures for correct harvesting, fixing, and submitting of Type 1 diabetic mouse pancreas for histological services and analyses.

#### **2. SCOPE**

This SOP shall be applied to all Type 1 diabetic studies that require histological services and analyses on mouse pancreas.

#### **3. RESPONSIBILITIES**

3.1 Managers and supervisors are responsible for making sure that technicians are properly trained and equipment is appropriately cleaned, maintained in good working order, and available as requested.

3.2 Laboratory personnel are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs.

#### **4. EQUIPMENT AND MATERIALS**

4.1 Ventilated work station to protect prosector from formalin fumes

#### **5. SAFETY**

5.1 Formalin: Avoid inhalation, ingestion, and skin contact. If skin contact occurs, flush affected area with water. Label all containers with a formalin warning label. Use formaldehyde spill kit for spills up to 1 liter. Call EHS for larger spills.

5.2 Work in well-ventilated area with appropriate personal protective equipment (e.g., gloves, etc.)

#### **6. PROCEDURE**

##### **Reagents:**

##### **1. General Laboratory Source**

10% Bleach

70% Ethanol

Phosphate buffered saline (PBS)

Anesthetic, as described in Institutional IACUC protocol

10% neutral buffered formalin (NBF)

1x HBSS

## 2. Thermo Fisher

10% neutral buffered formalin (NBF)

cat# 23-245-685

### Equipment:

Ventilated Work station to protect prosector from formalin fumes

Gloves and laboratory attire (e.g., lab coat, apron, etc.)

Cutting board

Serrated, blunt-ended forceps

Fine, blunt-ended scissor

Tape or pins to immobilize mouse on cutting board (optional)

Specimen containers

Cassetts with lids

Biopsy sponges

Weighing scale

### **A. Necropsy:**

1. Label specimen cups and cassettes with animal ID and relevant information before beginning.
2. Anesthetize and euthanize the mouse according to IACUC protocol of your institution.
3. Optional: weigh the mouse for total body weight; record the weight in grams.
4. It is helpful to always **orient animals in the same direction**, e.g. head up or head right, so that the side of any lesions can be recalled accurately. Tape or pin down limbs and wipe down fur with 70% ethanol.
5. Collect maximum attainable blood volume via cardiac puncture. Palpate to locate the end of the sternum. Using a 1ml syringe, pierce the thorax beneath the sternum and slightly to the left of center (the mouse's left). Slowly pull the plunger back to create a vacuum. Blood should gradually fill the syringe. If blood does not begin to fill the syringe, withdraw the needle and re-approach from a slightly different angle.
6. Open the abdomen; start at the pubis, and continue through the sternum to open the thorax; continue past the upper thorax with skin incision to the chin.
7. Dissect out the whole spleen, being sure not to tear it. If using spleen only for cell isolation for flow cytometry, transfer the whole spleen to a 50 ml conical containing about 20 ml of 1x HBSS (Hank's Buffer). If fixing a section of the spleen, see step 12 below.
8. Identify the pancreas by **gently lifting** the spleen. One can either dissect each separately or use the spleen to help pull the pancreas away from the upper GI tract during dissection. Recover the entire pancreas including left (near spleen) and right lobes (near GI). Pancreas and pancreatic lymph nodes are pale and soft tissues are difficult to distinguish grossly, but present very distinct histology. Place all together.
9. **Weigh the pancreas for subsequent beta cell mass calculations.** Average pancreatic wet weight is about 120 mg.

10. Place the pancreas on a dry sponge in a cassette. Spread it out gently to flatten it in as compact an area as possible. One should not see any major "mounds" of pancreatic tissue, nor should there be excessive stretching. Use only one sponge. Close the cassette lid securely and place in fixative (formalin).

11. It is suggested, for best practice, to include a piece of the duodenum (small intestine) with the right lobe of the pancreas for orientation and identification purposes.

12. Optional: Trim the spleen by taking a center cross section or longitudinal sample. Avoid submitting areas of the spleen that have been crushed by the forceps. Place into cassette, close the lid securely, and place into fixative.

13. Dissect out the right and left femur and tibia being sure not to break the bones (separate at the hip and ankle joints to remove rather than cutting through the ends of the femur or tibia). Trim most of the tissue away from the bones and place in a 1.5ml safelock tube containing 750ul 1x HBSS.

13. When fixing specimens (ie. pancreas), fixation time is generally overnight at room temperature. Fix for no longer than 24 hours, to minimize over fixation.

14. For samples intended for immunohistochemistry, it is imperative to standardize the time in fixative so that animals are handled consistently between treatment groups.

15. End fixation by transferring cassettes to either PBS or 70% ethanol and stored at 4°C. The samples are now ready for processing and embedding. This is often done by the institution's histology core or by a participating Laboratory Member. Suggestions and tips for this part of the process are given in the next section.

16. Used formalin should be discarded as hazardous waste.

#### **Additional Information:**

This protocol is adapted from the Standard Operating Procedure (SOP attached) for the Univ. of Florida College of Medicine Department of Pathology, Immunology and Laboratory Medicine Molecular Pathology Core. The purpose of this SOP is to outline proper procedures for correct harvesting, fixing and submitting of Type 1 diabetic (T1D) mouse pancreas for histological services and analyses. This SOP is applied to all Type 1 diabetic studies that require histological services and analyses on mouse pancreas. It is being presented here as a resource for those interested in doing immunohistochemical studies on mouse pancreas and diabetes.

It is recommended that the tissue sections be visualized using the Aperio system. Image analysis can be conducted on scanned slides to determine fractional insulin area (FIA). If pancreatic weight was obtained at necropsy, multiply FIA by the pancreatic wet weight to obtain beta cell mass. Insulinitis can be scored on H&E stained slides using the following scale:

0 = no infiltrates

1 = periislet infiltrates

2 = intraislet infiltrates to 3 = intraislet infiltrates >50%

4 = nonislet infiltrates (optional) The average scoring per animal can be expressed as percentage of 100 or by number of islets with each score.

Insulinitis will be scored based on the following scale:

0 = no infiltrates

1 = peri-islet infiltrates

2 = intra-islet infiltrates to : 50%

3 = intra-islet infiltrates > 50%

4 = non-islet infiltrates (optional, does not refer to islet-associated inflammation)

The scores from different levels can be averaged for each animal and expressed as percentage of 100 or by number of islets with each score.

## **7. REFERNCES**

7.1 RENI Tissue trimming guide: <http://reni.item.fraunhofer.de/reni/trimming/index.php>

## **8. RELATED DOCUMENTS**

## **9. REVISION HISTORY**