

STANDARD OPERATING PROCEDURE

Pre-clinical Consortium on Combination Therapies for Type I Diabetes

Document #: SOP-ITN5P-004

Supersedes: N/A

Applicable to: ITN Project 5P

Version #: 1

Effective Date: August 20, 2015

Category: Mechanistic Analysis

Title: Collection and Freezing of Fresh NOD Pancreas Tissue in OCT for Cryostat Sectioning

INTRODUCTION/PURPOSE

Tissue for freezing should be frozen as promptly as possible after cessation of circulation to avoid morphological distortions and damage due to tissue drying artifact, autolysis, or putrefaction.

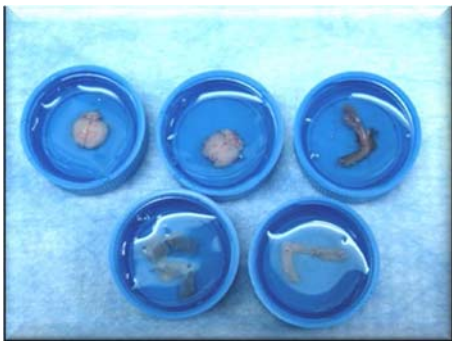
MATERIALS

Protective cold and water-resistant gloves
Cryomolds (labeled)
Aluminum foil (labeled)
Specimen bags (labeled)
Petri dishes or weigh boats (labeled)
OCT

Bucket containing crushed dry ice
Isopentane
Metal Spatula
12-inch metal forceps
Covered foam cooler containing crushed dry ice

METHODS

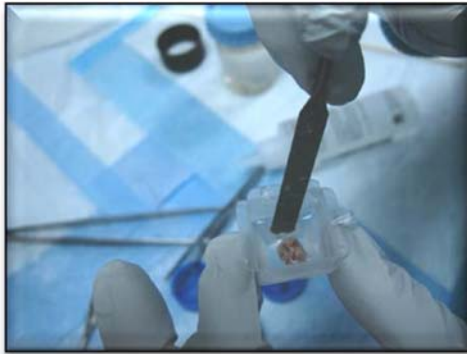
- Pour Isopentane into the bucket of crushed dry ice to create a slurry (at least 10 minutes before freezing sample).
- Add OCT to a labeled petri dish or weigh boat, place freshly dissected pancreas tissue in the OCT, and cover with additional OCT (allow tissue to “acclimate” to OCT for approximately 2 minutes).



- Add fresh OCT to a labeled cryomold (just enough OCT to cover the tissue) **AVOIDING BUBBLES.**



- Transfer pancreas to the cryomold and orient the tissue (try to spread out flat without tearing), keeping in mind that the bottom of the cryomold will be the sectioning surface.



- If needed, add additional fresh OCT to ensure that this tissue is covered.
- Using 12-inch forceps, **SLOWLY** lower the cryomold into the cold isopentane slurry (if the sample is dropped into the isopentane too quickly the OCT may crack or form bubbles). If necessary use spatula to ensure correct orientation of tissue.



- If a small amount of unfrozen OCT remains (droplet sized) transfer sample to covered foam cooler of dry ice and proceed with next sample (this avoids block cracking). Store on dry ice until all samples are frozen.



- Wrap individual samples in labeled foil, seal in a plastic bag, and store at -80°C .

