

Pathology Core Protocol



Protocol Section: Special Tissue Techniques-Manual

Policy No:

Protocol Subject: Somatic tissue snap freezing

Effective Date:

Date Reviewed: 24 February 2005

Date Revised:

Somatic Tissue Snap Freezing

This protocol describes a method for snap freezing somatic tissues for DNA/RNA analysis, enzyme biochemistry. The process of snap freezing involves ultra-rapid freezing of tissues by plunging directly into liquid nitrogen (LN2) creating in effect, a biochemical "freeze-frame". This leads to greatly enhanced RNA preservation, excellent DNA preservation and near instantaneous arrest of cellular biochemistry, important for studying enzyme profiles while minimizing autolytic biochemical artifacts.

Snap freezing of tissue samples is conducted during necropsy. In our lab, the left lateral hepatic lobe, and the entire tail are collected. Liver is a rich source of DNA; in addition this organ contains many enzymes important in clinical biochemistry. The tail tissue is collected for RNA and DNA as many labs have standardized their DNA/RNA extractions for tail tissue.

*** Liquid nitrogen safety training must be completed before this protocol is undertaken.**

1.0 Instruments

- 1.1 Sharp/blunt dissecting scissors
- 1.2 Forceps
- 1.3 1.8ml external thread cryovials (Wheaton)
- 1.4 4L Nalgene desk-top LN2 dewar
- 1.5 PPE for LN2 handling

2.0 Solutions

- 2.1 Liquid nitrogen (Praxair)
- 2.2 1x PBS without Ca Mg
- 2.3 70% Ethanol

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3.0 Tissue Collection and Processing for Snap Freezing

- 3.1 See protocol for Complete Necropsy of the Mouse for tissue isolation.
- 3.2 All instruments must be rinsed free of blood and tissue with 1x PBS, immediately followed by 70% ethanol to denature any contaminating DNA/RNA from previous animals. Wipe excess ethanol from scissors before using.
- 3.3 Liver sample is removed with liver *in situ* immediately before removal of the organ.
- 3.4 Grasp the left lateral hepatic lobe with clean forceps and excise with clean scissors taking care not to rupture the cholecyst.
- 3.5 Deliver sample to labeled 1.8ml cryovial, ensure cap is tightly sealed and plunge into liquid nitrogen.
- 3.6 At the completion of the necropsy, cut the tail into 1cm sections, place sections into a labeled cryovial and plunge into liquid nitrogen.
- 3.7 Register samples in Cryoarchiving Database and place them in indicated slots.

Issued by Lab Manager: _____ **Date:** _____

Approved by Facility Management: _____ **Date:** _____