

LABORATORY PROCEDURE: Freezing Tissue in OCT

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PURPOSE: To preserve surgically removed or biopsied tissue for histology and RNA

SCOPE: This procedure applies to all Tonsillectomy, Tonsil biopsy, Mouse spleen, Mouse kidney.

PRINCIPLE: Tissue is frozen in OCT upon arrival to laboratory. Tissue is then cut onto ICC+ slides

SAFETY PRECAUTIONS: Personal protective equipment such as: lab coat, gloves and glasses, should be used during the procedure. All surfaces and instrument should be wiped down with Rnase-Away if RNA will be used

NOTE: Pay particular attention to “**^HOT SPOT**” steps.

MATERIALS AND REAGENTS:

Supplies+Equipment

10 ml pipet (*VWR 89130-898*)
Scalpel (*Miltex 4-510*)
Biosafety carrier
Liquid nitrogen carrier
Cryomold (*Tissue-Tek 4566*)
Pipet aid
Refrigerator 4°C or Ice/bucket
-80C Freezer
Styrofoam bucket
8” forceps
Tissue forceps
kimwipes
100X20mm culture dish (*Falcon 353003*)
Specimen bag

Reagents

Rnase-Away (*Molecular Bioproducts 7002*)
OCT (*Tissue-Tek 4583*)
Liquid Nitrogen

REAGENT PREPARATION: None

REAGENT STORAGE: **Room Temp:** Rnase Away, OCT,

SPECIMAN STORAGE: Keep sample on ice until all components to freeze are ready. Once frozen, place immediately at -80C.

QUALITY CONTROL: Ensure that the Rnase Away is prior to expiration date.

PROCEDURE:

- Upon arrival to the lab, transfer the specimen cup to an ice bucket until ready to use
- **^HOT SPOT Rnase-Away** the surface of a biological safety cabinet and all utensils ie. forceps, pipeman, pipet aid ect.
- Remove the tissue with Rnase free tissue forceps and place in 100X20mm culture dish
- Divide with a scalpel if sample is large. Maximal dimension of 1cm sq.
- Take a section or a few small biopsy pieces and dab on the 3rd Kimwipe in the box (this should be Rnase free) Then place in cryomold and overlay OCT
- Retrieve liquid nitrogen in a proper container for transportation
- Pour liquid nitrogen into a Styrofoam container, about 2 inches deep.
- With long forceps take the cryomold and gentle touch the bottom of the cryomold to the LN without submerging it. let sit until **^HOT SPOT “Just”** frozen
- Remove and place in labelled ziplock specimen bag and place at -80C until ready for cutting

LIMITATIONS: No limitations

CALCULATONS: No calculations

INTERPRETATION: No interpretations

RESULTS REPORTING: The results are reported with Sample ID, Date, tissue per cryomold and # of cryomolds

TRAINING: Personnel will be trained by staff. Up to one time visual shadowing of staff member AND up to one time hands on training with staff member AND one or more times independent performance with successful completion of SOP.