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Version 2

615.1 URMHC HTC Non-Inflated Fresh-Frozen Embedded Lung and Associated Tissue V.2

DOI

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Protocol status: Working

We use this protocol and it's working

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Abstract

Purpose and Scope of the Procedure

- Rapid blocking, embedding and freezing of human lung tissue in no freezing media, 100% OCT or 5% CMC
- Rapid freezing of non-lung tissue in no freezing media, 100% OCT or 5% CMC



Materials

MATERIALS

✂ Brady FREEZERBONDZ Polyester thermal transfer printer labels **Fisher Scientific Catalog #22-500521**

✂ OCT (mold) **Catalog #Tissue-Tek 4583**

✂ Carboxymethylcellulose (CMC); 5% (w/w) CMC in DEPC H₂O **Catalog #Sigma C5678-1KG**

Worksheet 603.A.HTC_Whole_or_Partial_Lung_Processing

Biosafe surface and environment for manipulation of lung

■ Grossing Station, Biosafety Cabinet or Fume Hood

1. Gloves, face protection and clothing consistent with blood and body fluid precautions
2. Biohazards Disposal Bag
3. Red Sharps Container
4. Small and Medium Gauze Pads – multi-pack
5. Plastic Cryomolds
6. Scalpels and Trimming Blades with Handles; Forceps; Other Dissection Equip
7. Freezerbondz Labels and Printer
8. Standard Balance and Weigh Boats: Medium and Large
9. Rulers – metal 18 inch (45cm) and 12 inch (30.5cm)
10. 100% OCT (Tissue Tek)
11. 5% (w/w) Carboxymethylcellulose (CMC) in DEPC treated water
12. Flat ice buckets or large Styrofoam boxes partially filled with dry ice pellets
13. Flat metal pans
14. 200 proof (100%) ethanol or isobutane to create dry ice bath
15. Aluminum foil and labeling tape or freezer storage bags
16. Freezer Vials

Safety warnings

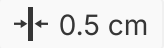
- ⚠ Working with human tissue: Personnel will adhere to safe work processes outlined in U.S. Public Health Universal Precautions Guidelines for use of human blood and body fluids. PPE will be used including lab coat, closed shoes and gloves. All activity will be behind shield of biosafety cabinet and/or with mask and safety glasses. Biosafety level 2 practices will be followed, and the work performed in the designated lab space that is covered by annually updated IBC approved protocol. All institutional biosafety measures are followed in any manipulation of these human tissues.

Fresh Tissue Frozen in Cryomolds

- 1 Slicing and blocking procedure should be accomplished in a grossing station or fume hood with the operator taking appropriate blood and body fluid precautions
- 2 Prepare dry ice-ethanol (or isobutane) bath by covering bottom of flat ice bucket or styrofoam box with dry ice pellets.

Pour in enough 200 proof ethanol to cover the dry ice and allow to boil. Once bubbling is stable, float a flat metal pan on the ethanol.

Add more dry ice and/or ethanol as needed to maintain rapid freezing of molds set on the metal pan.

Take care that ethanol doesn't splash over onto tissue in cryomolds.
- 3 Keep the surface of the lung lobes being processed moist and cool at all times
- 4 Select lobe or partial lobes for fresh embedding and freezing
- 5 Place fresh lobe on cutting surface and section tissue into slices and further into blocks (Our standard is approximately 1cm x 1cm x  0.5 cm thick. See sectioning diagram for lobe in 604. URM C HTC protocol).
- 5.1 Work quickly to avoid warming of lung tissue.
- 5.2 Photograph resulting tissue sections as a map of the stored blocks. Include labels in photos to record work, location of blocks and processing method applied.
- 6 Suggest alternating slices of tissue between different embedding media. For example, a slice each to i-iii, then repeat:
 - i.Fresh Flash Frozen Block [FFb] (not embedded in any media) - note: may not withstand long freezing period
 - ii.Fresh OCT Frozen Block
 - iii.Fresh CMC Frozen Block
- 7 For OCT or CMC embedding: Fill the cryomolds with a thin layer of the appropriate embedding medium

- 8 Place the tissue in their respectively labeled cryomolds and position them to allow for small amount of embedding medium around the tissue
- 9 Gently press down on the tissue with forceps in an attempt to remove as much air as possible and make sure the tissue lays flat along the bottom of the cryomold during freezing so easier to face block for sectioning
- 10 Place cryomolds containing tissue on metal pan in dry-ice ethanol bath and allow to freeze while filling the cryomold with freezing medium (OCT or CMC) to completely cover the embedded tissue.
- 11 While cryomolds are freezing, print out Freezerbondz labels for each block and place label on middle of a piece of aluminum foil.

Applying the label to the foil prior to wrapping the cold blocks promotes better adhesion and reduces any warming of block while wrapping.
- 12 Once frozen, quickly wrap each corresponding block with the correctly labeled aluminum foil and return to the frozen metal pan to keep frozen until transferred to -80°C freezer
- 13 Collect wrapped cryomolds into labeled freezer bags, place in cold labeled freezer boxes and store in -80°C freezer

Cryosectioning for Assays

- 14 The tissue-containing cryomolds can be sectioned as appropriate for the assay to be performed.
8-10 µm cryosections placed on glass slides for chemical stain as for H&E or low-plex immunofluorescent antibody staining.
40 µm cryosections tend to curl while being sectioned. One or more 40 µm "curls" can be placed in an RNase-free cryovial and stored at -80°C or shipped on dry ice for single nuclei isolation as in dx.doi.org/10.17504/protocols.io.ufketkw. The number of sections used is dependent on desired yield. For lung ~ 10 curls of ~ 1×1 cm tissue cryomold yields ~150-200K nuclei.
Alternating serial sections of 10 µm and groups of 10 × 40 µm "curls" are used to document the histology of the tissue from which nuclei are isolated.
- 14.1 See dx.doi.org/10.17504/protocols.io.3byl4j91jlo5/v1 for further instructions re: cryosectioning fresh frozen embedded lung tissue



Flash Frozen Tissue for homogenates / RNA Isolation

15 These tissue portions of Lung, Thymus, Spleen and Lymph Node are intended for processing for total RNA and for protein homogenates.

The tissue samples are sectioned into 0.25-1.0 cm³ portions and placed in properly Freezerbondz labeled freezer vials.

15.1 The weight of the collected tissue is recorded.

15.2 The freezer tubes containing the individual tissues are then frozen over liquid nitrogen and placed at -80°C for storage.

Recording and Analysis of Results

16 Complete worksheet and virtual freezer inventory

17 Correctly store photographs of grossing and blocking in Database