Nov 14, 2021

617.1 URMC HTC BSL2+ Non-Inflated Fresh-Frozen Embedded Lung and Associated Tissue

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dx.doi.org/10.17504/protocols.io.bjtsknne

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

LungMap2 Consortium 1

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Purpose and Scope of the Procedure
- Safe work with unfixed potentially or known SARS CoV2 / COVID-19+ infected tissue
- 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

DOI

dx.doi.org/10.17504/protocols.io.bjtsknne

Gloria S Pryhuber, Heidie Huyck, Lisa Rogers, Cory Poole 2021. 617.1 URMC HTC BSL2+ Non-Inflated Fresh-Frozen Embedded Lung and Associated Tissue. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bjtsknne

National Heart Lung and Blood Institute
Grant ID: U01HL148861-02S1

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OCT, CMC, rapid freeze, lung, COVID-19, SARS CoV2

protocol ,

Aug 17, 2020

Nov 14, 2021

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Scientific Principles:

1. CDC. “COVID-19 Personal Protective Equipment (PPE) for Healthcare Personnel.” *Centers for Disease Control and Prevention*, 1 Jan. 2020, www.cdc.gov/coronavirus/2019-ncov/downloads/COVID-19-PPE.pdf.
2. WHO. “Personal Protective Equipment for COVID-19.” *World Health Organization*, World Health Organization, 5 May 2020, www.who.int/medical_devices/priority/COVID_19_PPE/en/.
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MATERIALS

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

☒ OCT (mold) **Contributed by users Catalog #Tissue-Tek 4583**

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

Worksheet 603.A.HTC_Whole_or_Partial_Lung_Processing
Biosafe surface and environment for manipulation of lung

- Biosafety Cabinet or Grossing Station

1. N95 masks, face shields, double gloves, shoe covers, hair nets, sleeve covers, and labcoat for PPE, all consistent with recommended PPE for SARS-CoV2 / COVID-19 work
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. Cryovials with red cap inserts
17. Small biohazard stickers

Working with potentially or known SARS CoV2/COVID-19+ human tissue: Personnel will adhere to safe work processes all consistent with recommended PPE for COIVD-19 work. PPE will be used including N95 mask, face shield, lab coat, closed shoes and double gloves, shoe covers, hair net, and sleeve covers. 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 designatelabspace 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

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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 ehtanol 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 lobes 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 1cmx 1cm x **0.5 cm** thick. See sectioning diagram for lobe in 604. URMC 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

In the case where only 1 lobe is received, slices are taken for Cell Dissociation, Fresh Frozen Biopsy (in cryovial) and Formalin Fixed Paraffin Embedded Samples and fresh frozen blocks in OCT with potential postfixation in paraformaldehyde.

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

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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 along with a small biohazard sticker.

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

Flash Frozen Tissue for homogenates / RNA Isolation

14 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 cm^3 portions and placed in properly Freezerbondz labeled freezer vials.

14.1 The weight of the collected tissue is recorded.

14.2 The freezer tubes containing the individual tissues are then frozen over liquid nitrogen and placed at -80^0C for storage.

Recording and Analysis of Results

15 Complete worksheet and virtual freezer inventory

16 Correctly store photographs of grossing and blocking in Database

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