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Case Processing SOP for Lymph Nodes

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We use this protocol and it's working

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Abstract

The purpose of this Standard Operating Procedure is to outline procedures for processing and storing lymph node tissue received for HuBMAP consortium assay and analysis.

Guidelines

This SOP will be applied to all human donor lymph node tissues received for use in HuBMAP applications. Responsibilities: Managers and supervisors are responsible for assuring that technicians are properly trained and equipment and facility are maintained in proper working order. Laboratory personnel are responsible for reading and understanding this SOP and its related documents and to perform these tasks in accordance with the SOP. They are also responsible for following individual laboratory best practices and safeguards.

Materials

1. Sterile dissecting instruments (forceps, scissors, and scalpels)
2. Dissection boards with grids, sterile gauze sponges/paper towels
3. Ruler in millimeters
4. Dissection matrix 1cm X 1cm (Electron Microscopy Sciences Cat. No. 69010) optional
5. Centrifuge tubes (50 ml)
6. Dulbecco's Phosphate Buffered Saline (D-PBS), Mg2+ Ca2+ free (Invitrogen, Cat. No. 10010- 023), store at 4°C
7. Complete culture media (DMEM/F12 50/50 with L-Glutamine (Corning Cat. No. 10-090-CV + 10% Fetal Bovine Serum (Corning, Cat. No. MT35016CV) + 1x Antimycotic/Antibiotic
8. Uni-cassettes (Tissue-Tek®)
9.Macro Cassettes (ThermoFisher)
10. 10% neutral buffered formalin (NBF) in specimen container
11. O.C.T. ™ compound (Tissue-Tek®) and cryomolds, aluminum foil
12. Pipettes and sterile filter tips (200 µl, 1000 µl)
13. Serological pipets and pipet controller
14. 4% PFA (26ml d water + 4ml 10X PBS pH 7.4 without Mg or Ca + 10ml 16% paraformaldehyde EMS cat# 15710 for every 40ml of total volume needed)
15. Dry ice and ice bucket, 2-Methylbutane with dry ice in ice bucket and long forceps
16. Liquid Nitrogen in dewar, freezer gloves
17. Permanent markers, pencils
18. Foil squares (5cm)
19. 70% ethanol
20. 30 % Clorox bleach (6% Sodium hypochlorite)
21. Tissue waste container for formalin
22. Sharps container
23. 24 well cluster dish
24. 4 compartment + micromesh cassette (simport scientific)
24. Cryomolds (Tissue-Tek)

Troubleshooting

Safety warnings

1. Use universal safety precautions when handling human samples and employ personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron).
2. Perform dissection steps in a bio safety hood.
3. Follow chemical safety procedures and dispose of waste tissues in accordance with specific jurisdictional guidelines.
4. Handle sharps (e.g., scalpels, blades, glass) carefully and dispose of appropriately.
5. Dry ice and liquid nitrogen can cause freeze burns, handle carefully and use appropriate gloves.

Before start

Prepare 4% Paraformaldehyde in 1xPBS, place 350ml in a lidded speciman container.
Place 350ml NBFin a lidded specimen container.
Half fill the wells of a 24 well cluster dish with cold D-PBS and hold on wet ice.
Have liquid nitrogen and dry ice/methylbutane slurry on hand.
Assemble prefered dissection tools, gauze wipes and prelabeled cassettes and molds in biosafety hood.
Nest a metal pan half filled with D-PBS in wet ice to contain the organ until dissection begins

1 The tissues received will be identified as follows: Lymph Node (LN)

2 Sample prepared from tissues will be identified using the following nomenclature, abbreviations, and formats: Formalin Fixed Paraffin Embedded---FFPE (paraffin block)
Formalin Fixed Frozen Embedded---Fixed OCT Block
Fresh Frozen Embedded---Fresh OCT Block
Fresh Tissue piece cut to size---Tissue (in 4% PFA or complete medium in 50 cc conical)

3 Case Number Assignment: HuBMAP donor tissues will be assigned case numbers adhering to the following template: two digit year identifier, hyphen, sequential 3 digit number beginning with 001.

4 Labeling Samples:
a. Cassettes for paraffin embedding and Fixed OCT block fixation step
i. Generate label using cassette printer or print legibly in pencil
ii. Line #1: Case ID + Sample type abbreviation
iii. Line #2: fixative used
b. Cryomolds for fixed or fresh frozen OCT blocks
i. Print on mold legibly using permanent ink, apply printed label to bottom
ii. Line #1 as for cassettes
iii. Line #2 as for cassettes + FX if prepared to receive fixed frozen tissue
c. Dishes, foil wraps and containers (culture, petri or conical)
i. Print legibly using permanent ink or apply printed label
ii. Case ID + Sample type abbreviation (Table 1)

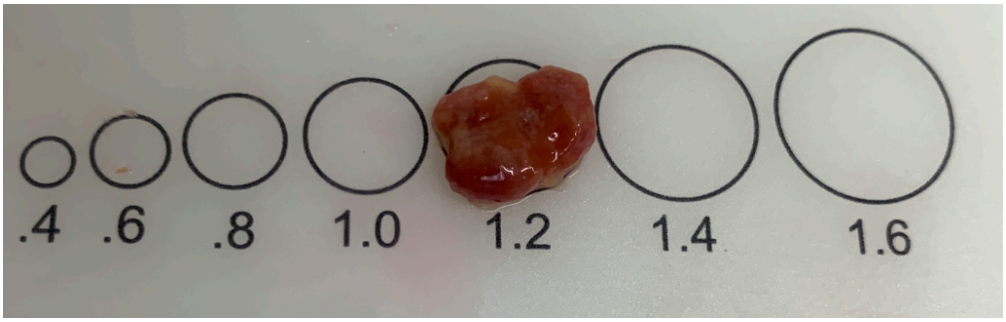
5 Processing Records:
a. Tissue handling data will be recorded on the Case Worksheet form.
b. Required fields during case processing include case identification, date received, any shipment or packaging anomalies, processing date and time (start and end), staff, tissues received, aliquot types and numbers, measurements and tissue quality notations.
c. Photo documentation should be employed to verify harvests are in keeping with guidelines.

6 Tissue Dissection:
a. Using sterile instruments, collect one lymph node of roughly 5 mm in size.
i. Place in individual RNAse free, 1.5 ml cryotubes and flash freeze in liquid nitrogen. Store at -80C for Quality Control analysis.

b. Dissect individual LNs away from fat. Place each in a single well of a 24 well cluster dish containing ice cold 1X D-PBS until collection of all lymph nodes is completed.
i. A minimum of twelve lymph nodes (or pieces) are required for a complete harvest.
ii. D-PBS volume should completely cover the lymph nodes

c. Remove any residual fat or connective tissues from each LN. Measure circumference using grid provided on dissection board and record size. Lymph nodes larger than 7 mm can be bisected if needed.

i. Photo document.



d. Assign lymph nodes to the various applications/fixations and record on worksheet.

e. Cassette tissue for three paraffin blocks using two medium LNs or three small LNs. Tissue must fit in a maximum area of 1 cm x 1 cm.

i. Place two cassettes into a container of NBF, the other into 4% PFA, as designated on the cassette labels. The NBF cassettes are reserved for Imaging Mass Cytometry and CODEX modalities.

Use a fixative volume of at least 20 mls per cassette added.

*cassettes are moved to 70% ethanol at 20-24 hours of fixation, and will be processed within 3 days.

f. Fix tissue for one fixed frozen OCT block using one to two medium LNs or three small LNs . Place (in a labeled cassette) into a container of 4% PFA. Use a fixative volume of at least 20 mls per cassette added.

i. Complete processing and embedding following SOP:
https://dx.doi.org/10.17504/protocols.io.basniede

g. Make one fresh frozen OCT block from one medium LN or several small LNs. Quickly blot excess moisture on a clean kimwipe or gauze and submerge tissue into an OCT filled labeled cryomold.

ii. Complete processing and embedding following SOP:
https://dx.doi.org/10.17504/protocols.io.bcwsixee
Hold and transport prepared blocks on dry ice.

h. Place 3 three LNs into labeled cassettes and emmerse in 4% PFA for use in CLARITY analysis. Use a ratio of at least 20 volumes of fixative to volume of tissue. Fix tissue for 20-24 hours at room temperature with gentle agitation.

* Tissue will be transferred into hydrogel solution and incubated 5 days at 4C on a rocking platform.

i. Obtain three LN to be used for 10x analysis.

i. If 8mm or larger in size, mince the tissue into small (5 × 5 mm) pieces. Transfer each lymph node into a 50 ml tubes containing 25 ml complete DMEM/F12 media. Store tissue at 4C until released for use or further isolation.

j. If the tissue is selected to be used for collaboration projects indicate protocol B on the worksheet.

i. Perform protocols as provided by the requesting lab(s).

7 Sample Archiving:

a. All materials obtained for this program will be inventoried by the University of Florida Tissue Mapping Center (UF TMC) and archived in the Organ Processing and Pathology Core (OPPC) until use.

b. Samples will be released for use or shipped to collaborator(s) upon request by the Principle Investigator.

8 Processing Worksheet for Lymph Nodes

Document

NAME

SOP Appendix for Lymph Node

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Preview