HuBMAP: Embedding Fresh Frozen OCT Samples

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1 Works for me

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

ABSTRACT

The purpose of this Standard Operating Procedure (SOP) is to outline procedures for the OCT embedding of HuBMAP fresh frozen specimens.

GUIDELINES

- Managers and supervisors - are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.
- Laboratory personnel - are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs.

MATERIALS

NAME	CATALOG #	VENDOR
Tissue-Tek® O.C.T. Compound, Sakura® Finetek	25608-930	Vwr
Tissue Tek Cryomold (25mmx20mmx5mm)	25608-916	
2-Methylbutane	O3551-4	Fisher Scientific
Ice / Dry Ice Bucket (EVA Foam)	03-395-152	Fisher Scientific

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Tissue Tek Cryomold (25mmx20mmx5mm)	25608-916	
Tissue-Tek® O.C.T. Compound, Sakura® Finetek	25608-930	Vwr

SAFETY WARNINGS

Avoid direct skin contact with dry ice.
Methylbutane should be collectedd as hazardous waste.

BEFORE STARTING

- Ensure you have proper scalpel blades, forceps, and your personal preference of gauzes/wipes.
- Embedding can be a messy process, to protect your clothes it is best to wear a lab coat or apron.
- Gloves are required when working with human specimens.

1 Collect tissue specimen from the designated common coordinate framework sites.
(See case processing SOP diagrams)

Appendix for Spleen SOP
by Marda Jorgensen

SOP Appendix for Spleen
by Franchesca Farris

PREVIEW

SOP Appendix for Thymus
by Franchesca Farris

PREVIEW

2 Prepare a pre-labeled Cryomold

Tissue Tek Cryomold (25mmx20mmx5mm)
Catalog #: 25608-916

and fill it half way with OCT compound.

Tissue-Tek® O.C.T. Compound, Sakura® Finetek
by Vwr
Catalog #: 25608-930

3 Gently blot tissue piece on dry gauze to remove any excess moisture.

4 Place the tissue into the OCT-containing Cryomold, maintaining tissue orientation.

5 Using forceps, push the tissue lightly to the bottom of the Cryomold to secure it.

6 Prepare dry ice/methylbutane slurry for freezing OCT blocks.

6.1 Place 1-2 inches of dry ice pellets into the bolltom of an ice bucket or styrofoam box.

6.2 Add enough 2-methylbutane solution to cover the dry ice by roughly 5mm.

6.3 Place lid on freezing chamber and allow methylbutane to chill.
Chamber is ready when fog dissipates and bottom of bucket becomes visible.

7 Fill the mold with OCT, covering tissue completely.
Avoid bubbles.

8 Freeze the tissue in the Cryomold by resting it on the surface of the methylbutane slurry.
Use long handled forceps to transfer the mold onto the slurry.
As the OCT inside the Cryomold begins to freeze, lightly push the tissue into the bottom of the mold one last time.

9 When the OCT Cryomold is thoroughly frozen and opaque, wrap the mold containing the tissue in a pre-labeled aluminum foil square and store at -80° C in a freezer rack box.

Enter block location if Freezer Log for future retrieval.