HuBMAP: Embedding Fixed Frozen OCT Samples

Marda Jorgensen[1], Jerelyn Nick[1]

[1]University of Florida

Feb 24, 2020

1 Works for me

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

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 frozen fixed 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
KimWipes		Fischer Scientific
Tissue-Tek® O.C.T. Compound, Sakura® Finetek	25608-930	Vwr
16% Paraformaldehyde	15710	Fisher Scientific
D-Sucrose (Molecular Biology) Fisher BioReagents	BP220-1	Fisher Scientific
PBS Phosphate Buffered Saline 10X Solution Fisher BioReagents	BP399-1	Fisher Scientific
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
PBS Phosphate Buffered Saline 10X Solution Fisher BioReagents	BP399-1	Fisher Scientific
Tissue-Tek® O.C.T. Compound, Sakura® Finetek	25608-930	Vwr

MATERIALS TEXT

16% Paraformaldehyde
PBS 10x Solution
D-Sucrose
OCT
Tissue Tek Cryomold
Methylbutane
Dry Ice
Ice Bucket

SAFETY WARNINGS

- Use physical safety precautions when working with sharps (disposable blades).

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 highly suggested to protect your fingers from the spectrum of hot and cold one encounters during the process.

Reagent Preparation 1h

1 Prepare **1x PBS**

PBS is required to prepare reagents needed for this procedure. Dilute 10x PBS 1:10 in deionized water to make working 1x solution.

PBS Phosphate Buffered Saline 10X Solution Fisher BioReagents
by Fisher Scientific
Catalog #: BP399-1

2 Prepare a **4% paraformaldehyde solution.**

Paraformaldehyde is toxic. Use gloves, wear a lab coat and work in a fume hood.

2.1 Add 60ml of 100mM PBS to a sample cup.

60 ml PBS

Prepare containers to accomodate number and size of tissues using atleast a 1:20 tissue to fixative volume ratio.

2.2 Open 2 -10mL ampules of 16% paraformaldehyde stock.
Add contents to the 1x PBS in the sample cup, invert to mix.

20 ml 16% Paraformaldehyde Stock

2.3 Label containers, solution expires one week after being made. Store at 4° C if not immediately used.

3 Prepare **30% sucrose** solution

3.1 Combine 300g Sucrose with 1 liter of 1X PBS. Add stir bar and mix on a stir plate until dissolved.

300 g sucrose

1 L 1x PBS

3.2 Store at 4° C in labeled container. Check for contamination prior to use, no expiation date.

Tissue Preparation Procedureure 3d 8h

4 Collect tissue specimen from the designated common coordinate framework sites. 30m
(See case processing SOP diagrams)

SOP Appendix for Spleen
by Franchesca Farris PREVIEW

SOP Appendix for Thymus
by Franchesca Farris PREVIEW

SOP Appendix for Lymph Node
by Marda Jorgensen PREVIEW

-

5 Place tissue into pre-labelled cassettes maintaining orientation. 15m

6 Transfer the tissue into a 120mL specimen container, pre-filled with 80mL freshly prepared fixative (4% paraformaldehyde in PBS). 1d

Fix specimen for 24 hours at room temperature under mild agitation using a rocker.

24:00:00 In Fixative on Rocker **Room temperature**

7 Drain fixative (collect as hazardous waste) and wash the tissue ***three (3) times*** for 10 minutes each in 1X PBS at a volume that generously covers.
Invert several times during this process, or return to rocking platform.
00:30:00
1h

8 Infiltrate and cryoprotect the tissue with 30% sucrose.
4 °C in Sucrose/PBS Mixture 72:00:00
2d

8.1 Combine equal volumes of 30% sucrose and 1x PBS to form 15% Sucrose. Replace PBS wash with 15% sucrose and place at 4° C for 8 hours

8.2 Drain 15% sucrose and replace with 30% sucrose. Equillibrate tissue 4° C for at least 48 hours.
Tissue is stable in sucrose for at least one week.

Freeze the Blocks 30m

9 Prepare a pre-labeled Cryomold
1m

Cryomold®
Histology
Tissue Tek® 25608-916
25mm x 20 mm x 5mm

and fill it half way with OCT compound.

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

10 Remove the tissue from the cassette using foceps and quickly touch the tissue to a Kimwipe to remove external sucrose droplet from tissue surface.
1m

11 Place the tissue into the OCT-containing Cryomold, maintaining original tissue orientation.
1m

12 Using forceps, push the tissue lightly to the bottom of the Cryomold to secure it.
1m

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

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

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

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

14 Freeze the tissue in the Cryomold by resting it on the surface of the methylbutane slurry.
As the OCT inside the Cryomold begins to freeze, lightly push the tissue into the bottom of the mold one last time.

20m

15 Add additional OCT to cover the tissue completely and fill the Cryomold.
Allow the tissue to equilibrate in the mold for ten (10) minutes.

00:10:00 In Cryomold

16 When the OCT Cryomold is completely 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 in Freezer Log for future retrieval