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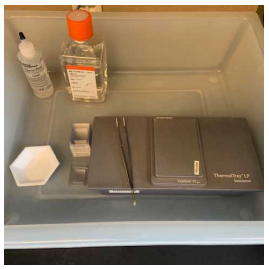
Formation of Optimal Cutting Temperature (OCT) Tissue Blocks for Cryosectioning



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We use this protocol and it's working

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Abstract

Optimal cutting temperature compound (OCT) is used to prepare frozen tissue blocks for cutting on a cryostat. This provides benefits for certain histological, immunofluorescent, and endogenous protein staining and detection. This protocol includes a PBS-to-OCT gradient to help tissue adhesion within the block ensuring the tissue does not separate from the block during sectioning. Also included are details on cryoprotecting fixed tissue using 30% sucrose to prevent excess water in the tissue from expanding and damaging the tissue when frozen.

Guidelines

This protocol describes the process of taking fresh or fixed tissues and taking them through cryoprotection and a PBS-to-OCT gradient for the creation of frozen blocks for cryosectioning. Improper technique while making OCT blocks can lead to tissue damage and ruined samples resulting in a loss of data and experimental setbacks. Properly prepared OCT blocks make cryosectioning easier and provide better results.

Materials

1. Phosphate Buffered Saline Sigma Cat# P38135 1X PBS (Phosphate-buffered saline)
2. Optimal Cutting Temperature OCT (Optimal Cutting Temperature compound) **Sakura Finetek Catalog #**4583
3. Sucrose sucrose **Sigma Aldrich Catalog #**84097
4. Dry ice
5. 6 well plate or medium weigh boats
6. Forceps
7. Large container safe for holding dry ice
8. Cryomolds

Equipment

Cryomolds

NAME

Tissue-Tek

BRAND

4557

SKU

https://us.vwr.com/store/product/4639407/tissue-tek-cryomold-molds-adapters-sakura-finetek

LINK

9. Biocision ThermalTray Cat: BCS-123

Equipment

ThermalTray	NAME
Thermal Tray for ULT	TYPE
Biocision	BRAND
BCS-123	SKU
https://ca.vwr.com/store/product/en/7747968/thermaltraytm-thermoconductive-modules-biocision#order	LINK

10. Biocision CoolSink XT9F Cat: BCS-536

Equipment

CoolSink XT96F	NAME
Small cold plate	TYPE
Biocision	BRAND
BCS-536	SKU
https://ecatalog.corning.com/life-sciences/b2c/US/en/General-Labware/Sample-Cooling-Systems-and-Accessories/CoolRack%C2%AE-for-Tubes/Corning%C2%AE-CoolRack%C2%AE-and-CoolSink%C2%AE-Plate-and-Reservoir-Modules/p/432070	LINK

11. Aluminum foil/Parafilm
12. 15 ml or 50 ml conical tubes

Safety warnings

Always follow BSL guidelines when handling fresh, unfixed tissue.

Wear proper PPE when handling dry ice.

All materials used with, or that come in contact with OCT or fresh tissue should be disposed of in biohazard waste or properly decontaminated after use.

Before start

Tissue can be fresh or fixed and cryoprotected through the use of 30% sucrose. Tissue should be fixed in accordance with experimental design and following proper fixation guidelines. Prepare 1:1 PBS to OCT solution the day before use, vortex or shake well to mix, and centrifuge after combining, or let sit overnight to remove air bubbles if necessary.

Fixed Tissue Cryoprotection (Fresh tissue start at Step 2)

1 **a.** Prepare a solution of 30% weight/volume sucrose in 1X PBS by adding 30g of sucrose to 100 ml of 1X PBS. **Increase this amount based on the number of fixed tissues that need to be cryoprotected.**

b. Prepare 1:1 PBS to OCT solution day before use, vortex or shake well to mix, centrifuge after combining, or let sit overnight to remove air bubbles. (Used in PBS-to-OCT Gradient step)

c. Place fixed tissue in adequate size containers (Usually 15ml or 50ml conical tubes) filled with 30% sucrose solution and place in 4C until the tissue sinks to the bottom of the tube. Highly fatty tissues may never sink but will still benefit from cryoprotection. **Failure to do so will result in an expansion of water when freezing which will severely damage the tissue.**

d. Once the tissue has sunk proceed to the PBS-to-OCT gradient.

PBS-to-OCT Gradient

2 **a.** Precool ThermalTray and Coolsink by filling large thermal safe container with dry ice and covering the ThermalTray and CoolSink, give 10-15 minutes for the trays to cool. **Be cautious and wear the appropriate PPE when handling dry ice.** (Used in Freezing Block step)

b. Using a 6-well plate, or other small containers such as medium weigh boats, fill the 1st with 100% 1X PBS, the 2nd with 1:1 PBS & OCT, and the third with 100% OCT.

c. Take tissue sample and place in 1st container with 100% 1X PBS. Using forceps gently move the tissue around in the PBS for around 30 seconds to 1 minute, removing any sucrose or other potential debris. **Do not leave tissue in PBS for long periods of time as it will undo the sucrose cryoprotection.**

d. Transfer tissue to 2nd container and repeat gentle movement in 1:1 PBS & OCT solution for 30 seconds to 1 minute.

e. Transfer tissue to 3rd container containing 100% OCT and gently move the tissue around for 30 seconds to 1 minute, ensuring that tissue is fully coated in OCT. **Use extra care when handling fragile tissue as the increased viscosity of OCT can cause tissue to separate or tear.**

f. Replace the reagents in the containers every 3 to 5 pieces of tissue, or when they appear visibly dirty. **Failure to use fresh reagents may result in cross-contamination between samples.**

Placing Tissue in Cryomold

3 **a.** While the tissue is in the 3rd container, prepare the cryomold by labeling and filling it with OCT. Fill the mold all the way with OCT regardless of tissue size.

b. Transfer tissue from 3rd container to cryomold and manipulate with forceps to achieve the desired orientation, specific orientation will depend on region of interest within the tissue. **Tissue should be as close to the bottom of the mold as possible**. Be careful to avoid air bubbles in the mold as this can lead to tissue damage. Remove any air bubbles with a transfer pipette. **Ensure that the tissue is fully covered in OCT**, add more OCT if necessary. It is okay if the OCT spills over the main region of the mold, this will not interfere with sectioning.

Freezing Block

4 **a.** Once tissue is in desired position, place the cryomold on top of the precooled ThermalTray. Once placed take the Coolsink and set it on top of the cryomold to cool the block from both top and bottom. Depending on the size of the block and the amount of OCT used the block should be solidified within 2 to 5 minutes. The OCT will be fully opaque once the block is solidified. **Moving the block before it is solidified may result in tissue being out of desired position.**

b. Once solidified the block within its mold can be placed on the dry ice surrounding the ThermalTray while steps 2c-4a are repeated with all remaining tissue. For fixed tissue ensure all samples go through sucrose cryoprotection before carrying out steps 2c-4a.

Storage

5 **a.** Once all blocks have been made, protect them from air with aluminum foil, parafilm, or a plastic bag, and store at -80C.