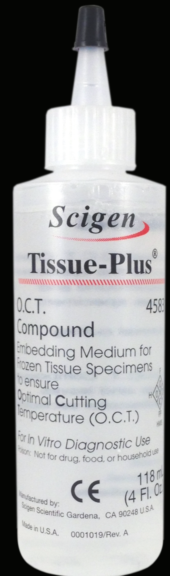
Tissue-Plus®

O.C.T. Compound

Optimal Cutting Temperature
Embedding Medium for
Frozen Tissue Specimens

User Manual

Tissue-Plus® O.C.T. Compound



Tissue-Plus® O.C.T. Compound is a water-soluble blend of glycols and PBA resins that provide a convenient specimen matrix for cryostat sectioning at temperatures of -10°C and below.

- Leaves no residue on slides during staining which eliminates undesirable background staining.
- Developed and optimized for microtome/cryostats and chromogenic IHC experiments.
- Medium is colorless and is supplied in a 118mL (4oz.) bottle.
- 50-75 specimens per bottle.
- Designed for cryostat sectioning or as a matrix for long term storage at -72°C.

Step by Step instruction for frozen sample preparation for histology assay in non-histopathology lab environment

Note: Before planning a frozen section project...

- Both fixed and unfixed tissue specimens may be frozen sectioned. If tissue is not preserved by a fixative, it is imperative to freeze it as quickly as possible after devascularization.
- Submitted specimens may be accepted either as fresh tissue on ice, formaldehyde fixed tissues in 30% sucrose, snap-frozen tissues, or OCT embedded frozen blocks.
- If the specimen is fixed with a formaldehyde fixative, it must be transferred into 15% sucrose in 1×PBS at 4ºC until tissue sinks. Then transfer the specimen into 30% sucrose in 1×PBS at 4ºC until tissue sinks. Submit the tissue to the core facility at the user's earliest convenience in 30% sucrose in 1×PBS or as frozen specimens embedded in OCT compound.
- On the day of frozen sectioning, place specimen on dry-ice or in liquid nitrogen and bring it to the core facility.
- Never freeze and thaw frozen tissue because ice crystal artifacts will occur. Remaining frozen tissues may be paraffin embedded through a special processing procedure.

Instructions are designed for those who work in non-histopathology lab environment to prepare frozen samples for histology assay.

Note: It is important to keep fresh tissues on ice and process them within 5-10 minutes of devascularization to preserve the nuclear acid and protein in the fresh tissue samples.

1. Grossing:

Specimens should be grossed in the same manner as paraffin sections. Avoid crushing artifacts by gently but firmly securing the specimen while cutting.

2. Size of the Specimen:

Smaller specimens are preferred, as they freeze faster. The specimen thickness is not as critical as in paraffin embedded specimens, they can be 1cm or a little more.

3. Fixation (optional):

The specimen may be fixed before or after grossing. Again, if the specimen is fixed with formaldehyde fixatives . It should be rinsed with 1×PBS for 10 minutes, 3 times, transferred into 15% sucrose in 1×PBS at 4°C until the specimen sinks, then transferred into 30% sucrose in 1×PBS at 4°C until tissue sinks.

If immunohistochemistry is required, perform an antigen retrieval procedure to expose epitopes due to the cross-link effect of aldehyde fixatives before performing immunostaining.

4. Rinse and Dry:

Remove excess liquid surrounding the tissue by absorption with a Kimwipe®, gauze, or paper towel, prior to freezing. Caution, this step is important to prevent liquid from forming ice crystals on the surface of the tissue and prevent tissue attachment to frozen embedding media (i.e. OCT compound). This procedure only takes a few seconds but it will make a significant difference in the sectioning process.

5. Snap-Frozen(optional):

Specimens may be snap-frozen on dry-ice or in liquid nitrogen when:

- They are collected over a period of time and cannot be processed simultaneously.
- There are too many specimens to process at once.

While freezing allows the researcher to postpone DNA/RNA/protein isolation, it is time consuming and makes subsequent RNA isolation a more laborious process. Ideally, specimens should be snap-frozen or placed in an appropriate reagent within 5 minutes from loss of vascularization to prevent the DNA, RNA and protein from degradation and preserve morphology.

Transfer the frozen sample into a cold, labeled cryo-vial with a screw-top lid. It is not recommended to put fresh tissue inside the cryo-vial first and snap-freeze the tissue in the vial; it will become difficult to take the tissue out of the tube later.

Powder dry-ice/cold isopentane in granular dry-ice/liquid nitrogen plus cryo-vial method provides excellent specimen integrity and a wide array of options for tissue analysis.

The vials may be stored in a liquid nitrogen storage tank or -80°C freezer for long-term storage.

6. Freeze Source:

An ideal freeze source should be able to freeze the tissue relatively quickly and evenly. The likelihood of ice crystals artifacts occurring decreases the faster the specimen is frozen; however, freezing tissue too quickly, such as submersing the tissue directly into the liquid nitrogen, will cause the tissues or blocks to crack, which will make them difficult to section. This occurs because the outer perimeter of the tissue begins to freeze quicker than the internal portion.

The following freeze sources are recommended for those who want to embed their specimens by themselves in a research laboratory without special frozen apparatus:

Cold (at least -80°C) isopentane/2-methylbutane/methanol/ethanol in dry ice/ liquid nitrogen or liquid nitrogen in a dewer flask with a relative wide opening which allows the user to put the embedding mold in the vapor phase of the liquid nitrogen easily. A metal freezing stage or disk may be used to combine with dry-ice/liquid nitrogen as an alternative freezing source. Using this method, cool down the metal blocks in -80°C freezer/dry ice/liquid nitrogen before using.

Note: freezing tissue on granular dry-ice or in the freezer is not recommended. These types of freezing sources do not provide even freezing or freeze the tissue quick enough and may cause freezing artifacts and desiccation of the tissue.

7. Embedding Unfrozen Tissue in OCT Compound

1) Place a few drops of OCT compound (depends on the size of the tissue to be embedded) onto the center of the bottom of a cryomold, a small thin plastic tray or a small weigh boat. Be careful to select the proper size embedding mold according to the size of the tissues to be embedded.

2) Place the unfrozen tissue specimen in the drops and orient. Make sure the side touching the bottom of the plastic tray, cryomold or weigh boat is the side you want sectioned. Gently push the tissue with forceps to ensure that the bottom surface of the tissue is oriented properly and flat against the bottom of the chosen embedding mold and orient the specimen in the center of the mold. *Carefully orient the sample properly; it is imperative for the demonstration of expected morphology site.*

3) Carefully add additional OCT into the mold until the specimen is completely covered, leaving no tissue exposed. If using a cryomold to embed tissue, be certain to completely fill up the mold.

4) Avoid the formation of air bubbles inside the OCT compound that may occur in the mold; remove any air bubbles that may form. This is important because the air bubbles create problems during sectioning.

5) Let the OCT compound settle for 15-30 seconds to allow the OCT to wet the surface of the tissue.

6) Place plastic tray, cryomold, or weigh boat with OCT covered specimen on the surface of the cold isopentane/2-methylbutane/methanol/ethanol or in the vapor phase above the liquid nitrogen with the flat side down using long forceps.

Caution: Do not freeze the tissue by submersing it into the liquid nitrogen, the blocks will crack making them difficult to section; this occurs when the outside of tissue freezes faster than the internal portion.

7) After freezing the OCT compound (approximately one minute), detach the OCT block from the plastic tray/weigh boat and wrap the OCT embedded block in foil and place in a labeled bag or vial.

8. Embedding Frozen Tissue in OCT Compound:

Embedding frozen specimens is similar to embedding unfrozen tissue, except that you must freeze the frozen specimen in OCT compound as soon as possible without any delay. Otherwise the frozen tissue will experience a freeze- thaw-freeze cycle, forming ice crystals which result in poor morphology due to the ice crystal artifacts.

9. Storage:

The frozen blocks may be temporarily stored in dry ice. Transfer the blocks to a liquid nitrogen storage tank (Years) or -80°C freezer (Months). From this point on, the specimen should never be thawed unless there is a specific requirement.