



USER MANUAL

WELCOME

1 | COLLECT MATERIALS

WARNING! ONLY PROPERLY TRAINED USERS SHOULD HANDLE CRYOGENIC FREEZING MEDIA, SUCH AS LIQUID NITROGEN AND DRY ICE.

NEVER ALLOW CRYOGENIC FREEZING MEDIA TO COME INTO CONTACT WITH SKIN. ENSURE PROPER SAFETY GEAR, INCLUDING GLOVES, GLASSES, AND PROTECTIVE CLOTHING IS EQUIPPED AT ALL TIMES WHEN HANDLING FREEZING MEDIA. ALWAYS USE FORCEPS WHEN INTRODUCING SEAL'N FREEZE TRAY INTO SEAL'N FREEZE BOX AND WHEN REMOVING SEAL'N FREEZE TRAY FROM SEAL'N FREEZE BOX.

TO PREVENT TIPPING, USE SEAL'N FREEZE BOX ONLY ON A LEVEL SURFACE AND AVOID QUICKLY SWINGING LID OPEN AND SHUT.

IN CASE OF FROSTBITE OR OTHER INJURY, OBTAIN MEDICAL TREATMENT IMMEDIATELY. PRODUCT MANUFACTURER IS NOT RESPONSIBLE FOR INJURIES OR MISUSE OF DEVICE.

Welcome to your new process for cryogenic tissue freezing. Welcome to processing multiple samples simultaneously. Welcome to the fastest technology in freezing. Welcome to free time to perform other tasks. **Welcome to Seal'N Freeze.**

The Seal'N Freeze box is intended for the cryogenic freezing (also known as "snap" or "flash" freezing) of animal or human tissue samples for use in cryostat sectioning. Follow the standard established procedures of your institution with respect to tissue sample harvesting and fixation prior to use of your Seal'N Freeze box.

Required materials:

- Seal'N Freeze box
- Seal'N Freeze tray(s)
- Forceps
- Marker for labeling tray(s)
- O.C.T. (Optimal Cutting Temperature) compound
- Gloves
- Safety glasses
- Cryogenic freezing media*

*For the freezing media, use either:

Liquid nitrogen

OR

Dry ice with alcohol

2 | PREPARE THE SEAL'N FREEZE BOX

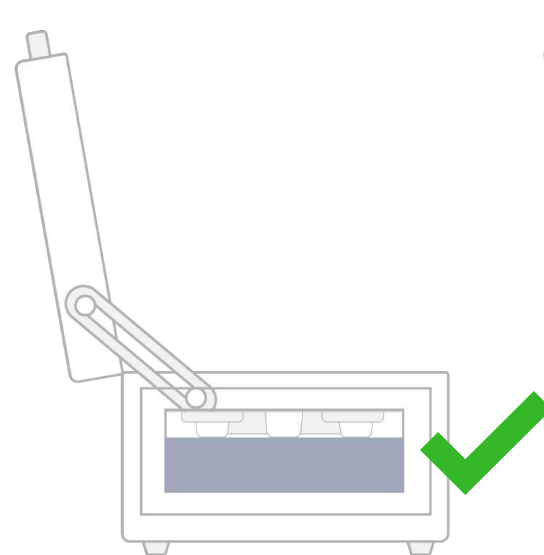
A) Fill the Seal'N Freeze box with 240mL of your selected freezing media; either:

Liquid nitrogen (LN2)

OR

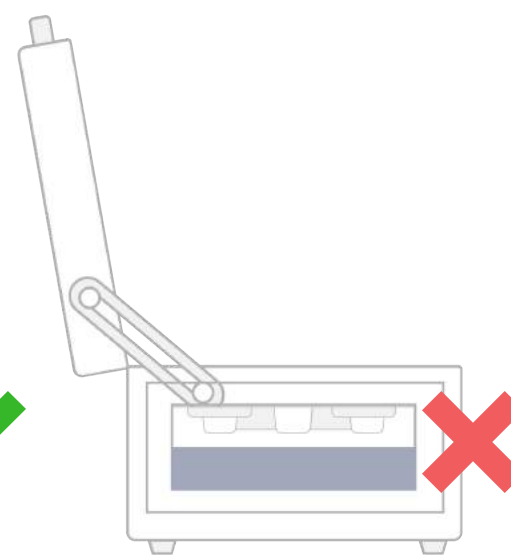
Dry ice with alcohol

The box should be filled to just below the inner ledge, allowing space for the Seal'N Freeze tray to rest properly on the ledge. The fill level should be high enough that the bottom of the tray touches the freezing media, but not so high that the tray cannot rest on the inner ledge.



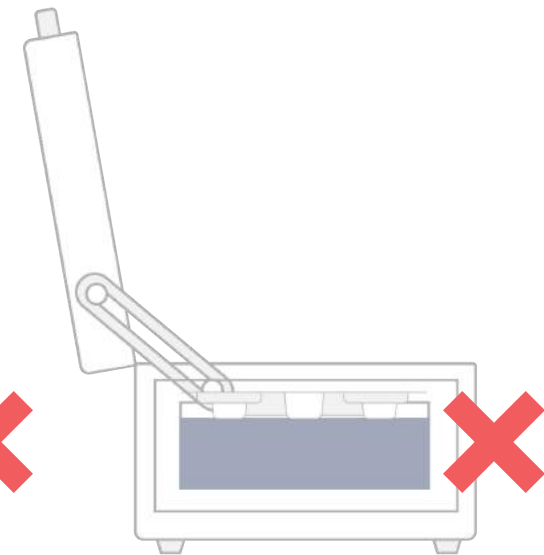
CORRECT

Tray is touching freezing media
Tray is resting on inner ledge



INCORRECT

Fill level too low
Tray not touching freezing media



INCORRECT

Fill level too high
Tray cannot rest on inner ledge

B) After preparing the freezing media in the Seal'N Freeze box, close the lid before preparing your Seal'N Freeze trays and tissue samples. This will allow the box to maintain optimal freezing temperature for an extended duration.

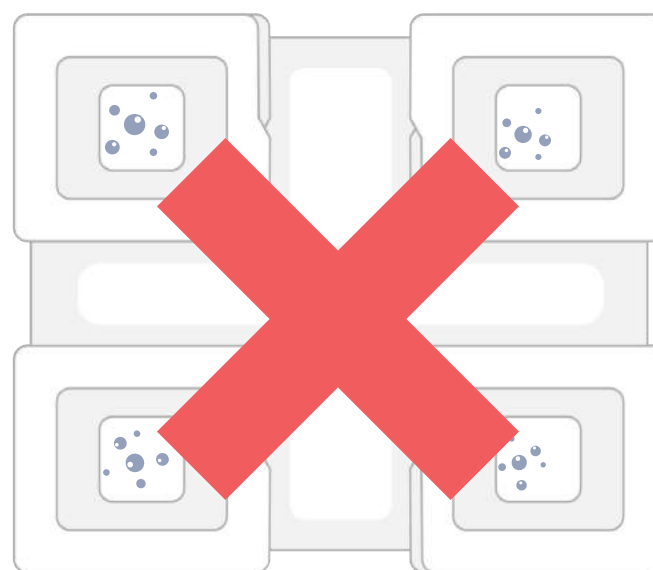
3 | PREPARE THE SEAL'N FREEZE TRAY

Each Seal'N Freeze tray contains four individual sections, allowing for the efficient processing of multiple tissue samples simultaneously.

- A) Before freezing your samples, use a marker to label each section of the tray with a unique ID for easy identification of the samples following the procedure.
- B) Fill each section of the tray with the O.C.T. (Optimal Cutting Temperature) compound of your choosing. Fill sections slowly to avoid formation of bubbles in the O.C.T. compound.



CORRECT
O.C.T. compound is smooth and free of bubbles



INCORRECT
Bubbles in O.C.T. compound

TIP: Forceps can be used to pop bubbles in the O.C.T. compound prior to introducing tissue samples into the tray.

4 | PREPARE THE TISSUE SAMPLES

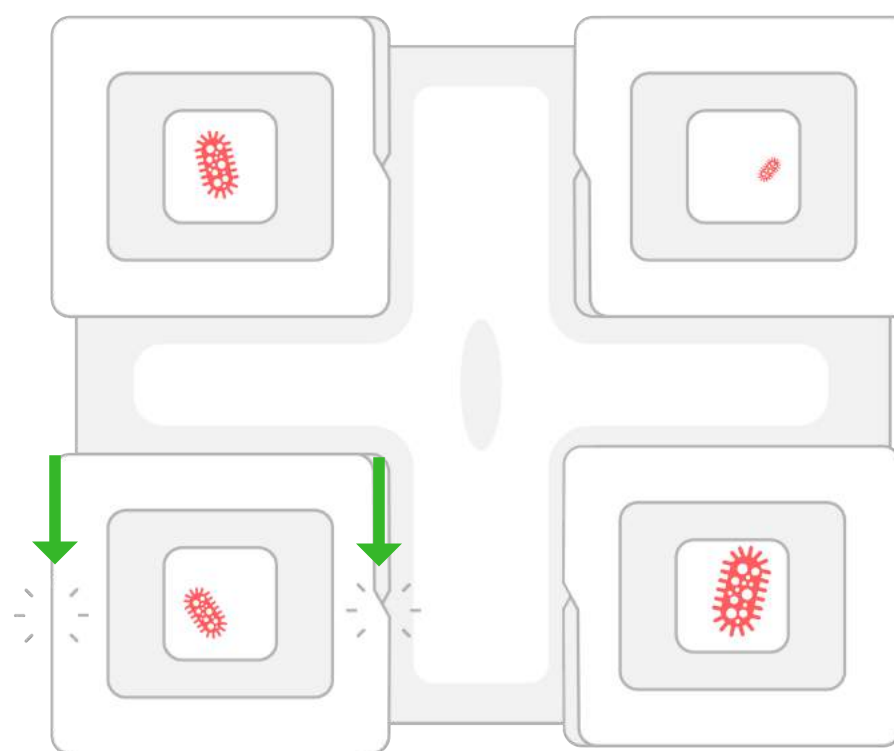
NOTE:

To avoid autolysis (self-digestion), drying, or putrefaction (decomposition), tissue samples should be frozen or fixed immediately following resection.

- A) Using forceps, carefully place each tissue sample into the O.C.T. compound of its own section of the Seal'N Freeze tray.

Tissue samples should be oriented so that the surface that you wish to examine is facing the bottom of the Seal'N Freeze tray. This bottom surface will be the first layer sliced by the cryostat machine.

- B) Seal each section by pressing firmly on opposite sides of the lid. Each lid will "snap" into place, indicating that it is completely sealed.

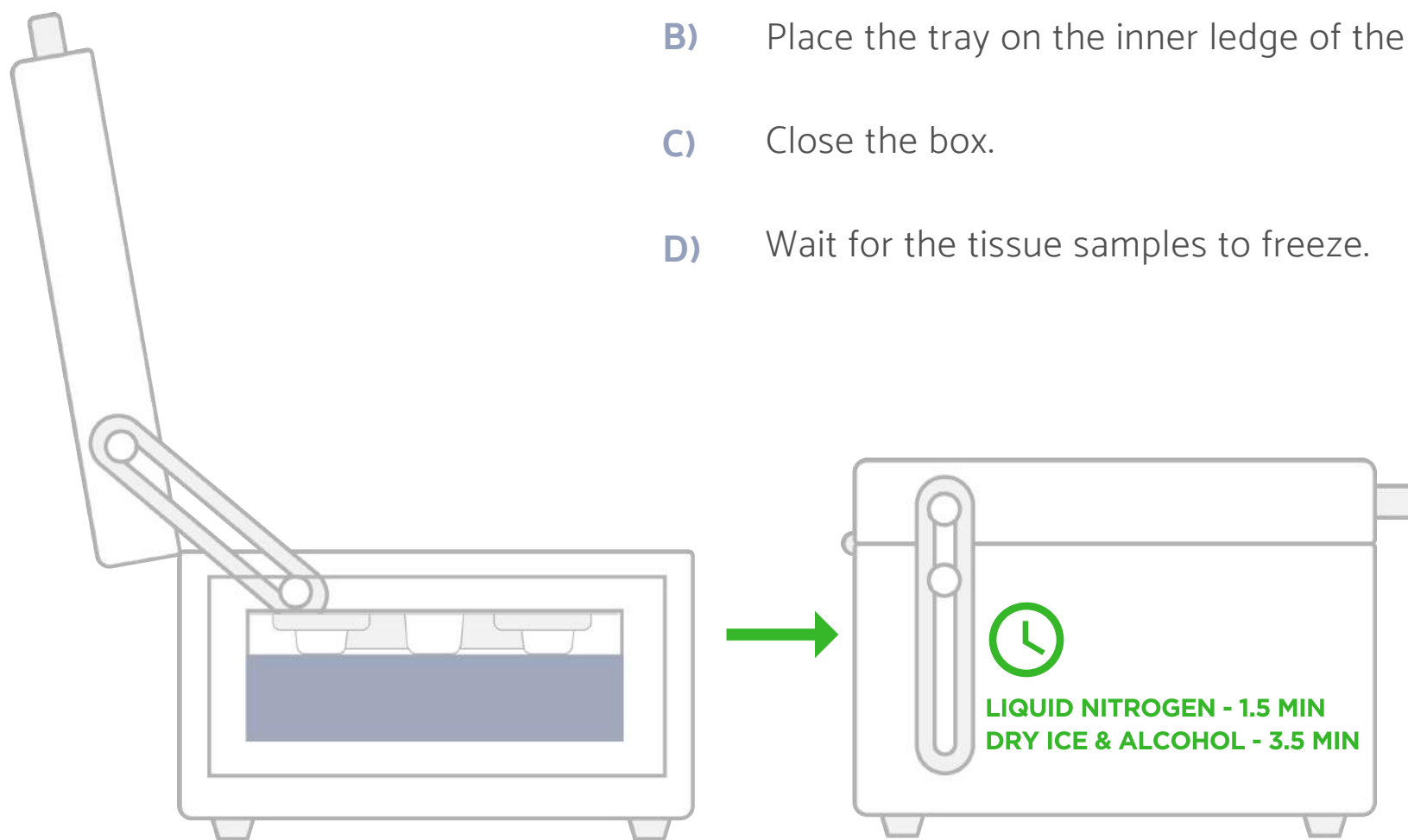


Press down firmly on opposing sides of the lid until it "snaps" into place.

5 | PLACE THE TRAY IN THE BOX

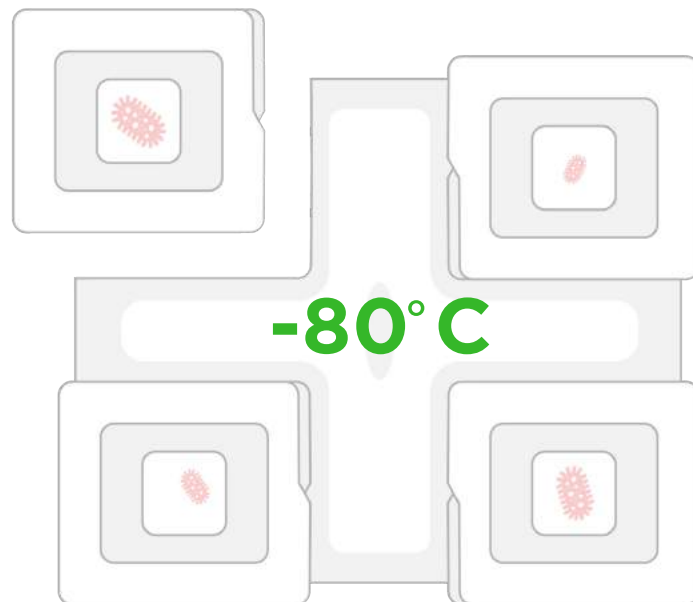
After preparing your tissue samples in the Seal'N Freeze tray and filling the Seal'N Freeze box with your freezing media, you are now ready to use the box to freeze your samples.

- A) Open the box.
- B) Place the tray on the inner ledge of the box.
- C) Close the box.
- D) Wait for the tissue samples to freeze.



TIP: Planning to freeze multiple batches? Start preparing your next four samples in a new Seal'N Freeze tray while you wait, streamlining the tissue freezing process.

6 | REMOVE THE TRAY



Tray design allows for easy fold-and-tear separation of each section. Store samples between -20°C and -80°C after freezing.

- A) Use forceps to remove the Seal'N Freeze tray from the Seal'N Freeze box, taking care not to come into contact with the freezing media.

The Seal'N Freeze tray is designed for easy separation of each section. Carefully fold and tear away from the "+"-shaped center connector to separate your samples. Appropriate protective gloves should be worn when handling the tray after freezing.

If necessary, frozen samples can be temporarily stored in a covered foam cooler of dry ice while you are processing additional batches.

Otherwise, samples should be stored in a freezer at -80°C until introduced into the cryostat chamber.

NOTE: If you are processing multiple batches, the next prepared tray of samples can be placed in the box immediately after removing the first batch.

CAUTION: When you are finished using the box, excess freezing media should be disposed of in accordance with your lab's standard safe handling procedures. Allow the surplus of freezing media to evaporate or sublimate inside a fume hood or other well-ventilated area. Never dispose of liquid nitrogen or dry ice in the sink, toilet, or garbage.



CLEANING AND CARE

The outer surfaces of the Seal'N Freeze box should be disinfected according to your laboratory's standard cleaning procedures. Avoid scrubbing the box with abrasive scouring pads or cleaners. Never use hydrochloric acid (muriatic acid) as it will lead to corrosion of the box's finish.



RE-ORDERING INSERT TRAYS

If the foam insert of your Seal'N Freeze box becomes damaged, contact info@sealnfreeze.com to order a replacement. The foam insert should not otherwise be removed from the box.

To re-order Seal'N Freeze trays, visit www.sealnfreeze.com or contact info@sealnfreeze.com.