SEAL'N FREEZE®
Protocols

Our Seal'N Freeze® Cryotray® consists of four molds designed for batch processing of multiple tissue samples simultaneously and/or easy separation of the individual molds. Sealable lids provide optimal protection for storage and ensure the uniform shape of each frozen sample.

The Seal'N Freeze® Box is intended for the cryogenic freezing (also known as 'snap' or 'flash' freezing) of tissue samples for use in cryostat sectioning. Its small, compact size provides an ideal form factor for bench-top freezing operations using various freezing media, such as liquid nitrogen or a slurry of dry ice and alcohol.

Recommended starting quantity of dry-ice and alcohol:

- 100g dry ice and 55ml of alcohol for large Seal'N Freeze® Cryotrays®
- 100g dry ice and 150ml for small Seal'N Freeze® Cryotrays®

PROTOCOL FOR EMBEDDING TISSUES IN O.C.T. COMPOUND

1. Label the sample ID on the surface of the cryomold with a permanent maker or a coded sticker.

2. Slowly add enough O.C.T. compound to support the tissue sample for positioning. Avoid air bubbles and overfilling. Be careful to select the proper size embedding mold according to the size of the tissues to be embedded.

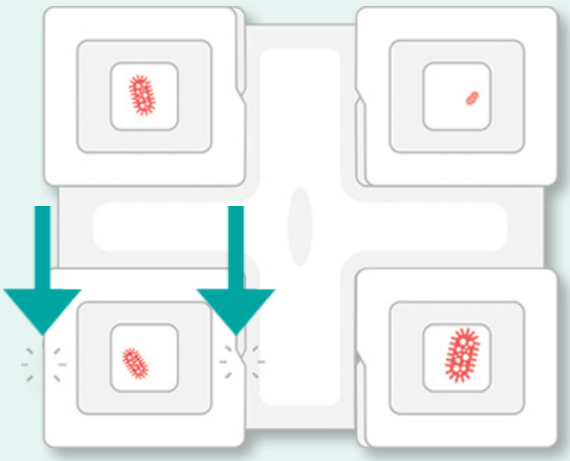
3. Place the tissue sample/samples in the O.C.T. drops and orient the tissue sample. Make sure that the side touching the bottom of the cryomold is the side you want to section first, which is important for the demonstration of proper morphology. Use tweezers to position the sample. If the sample is rather small, it can be easier to position the sample by manipulating the O.C.T. compound surrounding the sample rather than trying to move the sample itself. Remember that sectioning will begin from the bottom of the mold as you can see it in front of you when embedding. .

SEAL'N FREEZE

4. Carefully add more O.C.T. to the mold until the specimen is completely covered. None of the tissue should remain exposed.

5. Avoid air bubbles. Remove any accidental bubbles from the mold by driving them up and to the sides with forceps. This is important because the air bubbles will create problems when cutting sections. Avoid shaking O.C.T. bottle before use.

6. Let the sample settle for a few seconds to allow the O.C.T. to completely wet the surface of the tissue. Then close the cryomold lids. Seal each mold by pressing firmly on opposite sides of the lid. Each lid will snap into place indicating it is completely closed.



7. Follow instructions on the right side of this page for information on preparation of the Seal'N Freeze® Box for freezing. Place the closed Seal'N Freeze® Cryotray® with the sample inside, into the prepared Seal'N Freeze® Box by grabbing the mold at the central stud with long forceps. Please make sure the Cryotray is properly lined up with the slot in the box, and close the lid.

Liquid Nitrogen with Ethanol Freezing Protocol:

1. Use protective gloves, as well as eye and face protection.
2. Carefully pour 200ml of liquid nitrogen into the Seal'N Freeze® Box, let it settle and allow for the temperature to equilibrate. After a few minutes, check the level of nitrogen in the box. There should be just enough nitrogen in the box to allow the flat bottoms of Cryotrays® to be bathed in the nitrogen vapor phase without directly touching liquid nitrogen. Add liquid nitrogen as needed.
3. Place the Cryotray with samples into the tray slot of the Seal'N Freeze® box and close the box lid. Leave the Cryotray in the box for a few seconds to a few minutes, or until O.C.T. is totally solid.

Protocol for Dry Ice and Ethanol Freezing:

1. Use protective gloves. Use fume hood for operations with flammable liquids.
2. Add a scoop of dry ice which might fill 100ml volume in a glass beaker. Do not use large chunks; use pellets or granules instead.
3. Pour 55ml of ethanol (methanol, isopentane) into the Seal'N Freeze® Box for large Cryotray® (185 ml for small Cryotray®). When using the large Seal'N Freeze® Cryotray® be mindful of the volume of your dry ice to ensure that it fits correctly within the Seal'N Freeze® Box to prevent overflooding of the Cryotray® with alcohol.
4. Place the Cryotray with samples on top of the Seal'N Freeze® Box and close the lid.
5. Keep the Box closed for between 1 minute to 3.5 minutes, or until O.C.T. is totally solid (O.C.T. turns from clear liquid to opaque white solid upon freezing).

8. After hardening of the O.C.T. compound (it usually takes a few seconds to a few minutes depending on the size of the Cryotray®, sample and amount of O.C.T. used), take out the Cryotray®, wipe any remaining liquid from the bottom of the molds with a Kimwipe tissue and place it into storage.

9. **Storage:**

a) Store the samples frozen. Suggested short term storage temperature is -20°C. Suggested long-term storage temperature is -80°C and below. Please make sure to equilibrate the blocks to the cryostat cutting temperature before cutting sections. Storing blocks in liquid nitrogen further extends the shelf life of the samples.

b) From this point on, the sample should never be thawed unless you have a specific requirement to do so.

10. Individual molds in the Cryotray® can be easily separated from the rest of the tray if needed: carefully fold and tear away from the center connector.

Cleaning Seal'N Freeze® Box After Freezing

1. **After the freezing process, dispose of residual alcohol and dry ice in accordance with your local guidelines and regulations for waste handling. Leave the box open inside the fume hood until dry.**
2. **Alcohol may be collected and reused after de-gassing. Please make sure to de-gas it properly before storing in any closed container.**
3. **If liquid nitrogen is used, close the box lid after use, put the box away in a secure area to avoid any accidents, and allow the nitrogen to evaporate on its own.**

KLAREX® Health, LLC
PO Box 185755
Hamden, CT 06518 USA
klarexhealth.com