Pathology Services Core

Frozen Tissue Preparation

Disclaimer

We recommend submitting a sample-pilot study to test the morphological viability of your preparation before submitting actual test samples.

Freezing tissues (with the exception of muscle) requires the following materials, regardless of the method of freezing:

1) Cryomolds (plastic tissue molds specifically used for freezing tissues for histology)
    - Cryomolds come in an assortment of sizes

    - Choose the smallest size that will work for your tissue
    - Label molds with a sharpie, bend the corner to enable holding with forceps during freezing
2) OCT compound- Tissue Tek OCT Compound
    - This serves as a matrix to uniformly freeze the tissue as well as to support the tissue during cryosectioning

3) Long forceps
    - These will be used to hold the cryomold in the freezing solution
    - Ideally use non-curved long forceps to improve gripping the cryomold
4) Paper towels to blot samples dry prior to placing in cryomold
5) Pasteur pipette to help remove bubbles from OCT in the cryomold
6) Aluminum foil or small baggies to place frozen samples in prior to storage in -80
7) Dry ice in a container to place frozen samples in until they can be moved to -80

Freezing tissues in cryomolds is the same, regardless of how the samples are frozen. Detailed set-up methods for freezing follow this section.

1) Label the sample ID on the surface of the Cryomold with a cryomarker or permanent marker.
2) Bend a corner up to serve as a place to hold the mold with the forceps.
3) Place gently blotted tissue on a small dot of OCT in the cryomold – ensure it is flat and there are no bubbles on the cutting surface (the bottom face of the tissue which is against the cryomold base)
4) Cover the tissue to the top of the cryomold well with OCT. Before you begin filling the well, make sure there are no bubbles coming out of the nozzle. Bubbles result in gaps once the sample is frozen, which can result in poor sectioning (e.g., fragmenting, chipping).
5) Hold the mold using forceps by the bent corner and place mold only up to the top of the well into the freezing solution. DO NOT submerge the cryomold as the freezing solution will boil in the OCT medium, creating bubbles and tissue distortion.
6) Hold the mold in place until all the cryomedium is solid white – it will freeze from the outside of the mold in
7) Freezing time will depend on the size of the cryomold and tissue; wait until the cryomedium is completely frozen through (white opaque). Freezing starts at the edges and moves toward the center
8) If using liquid N2, remove sample to dry ice as soon as frozen. Retention in N2 once freezing is complete will result in the cracking of the block and/or the tissue, which complicates cryosectioning and reduces histology quality.
9) After freezing of the OCT compound, wrap the OCT embedded block in foil, placed in a labeled zip bag or labeled box, and store in a -80C freezer until ready to transport to the lab. OCT samples can be transported on dry ice in a Styrofoam container.

Freezing muscle tissue

1) Muscle samples should be frozen directly in the freezing solution without OCT
2) Samples may be oriented by placing a dot of OCT on cork and placing the muscle on the OCT to serve as a "glue"
3) Frozen muscle may be cut directly from the cork placed on the chuck
4) Ensure that the muscle is large enough to accommodate sectioning if adhered to cork
5) Place directly in freezing solution – liquid N2 freezing should be complete in less than 30 seconds. Beware prolonged freezing in liquid N2 → tissue will crack
6) Retain samples on dry ice until placement in -80 freezer.

DETAILED INSTRUCTIONS ON FREEZING SET UP OPTIONS

Options are listed below for freezing depending on what reagents you have access to (i.e., liquid N2, dry ice, and isopentane). Do not freeze tissues directly on dry ice.

Ideally, cryomolds are frozen either on a stainless steel block chilled in liquid nitrogen or in isopentane (2-methylbutane) chilled in liquid nitrogen.

Isopentane is used for tissue freezing because of its very low freezing and boiling temperatures – this allows tissues to be frozen at a very cold temperature in a uniform way. *However, isopentane is an extremely volatile and flammable liquid at room temperature and pressure.* For this reason, some labs may prefer not to use it.

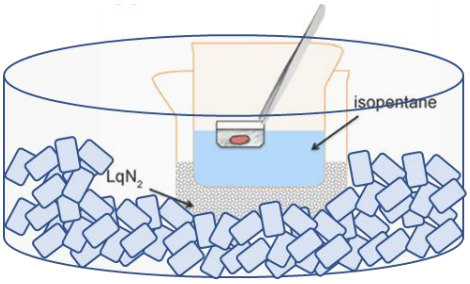
Freezing if you *only* have access to liquid N2:

1) The perfect freezing set up if you only have liquid N2 is to use a stainless steel block sitting nearly to the top in the liquid N2. Samples may be frozen directly on the chilled plate.
    - A relatively large amount of liquid N2 is generally needed, particularly if there are numerous tissues to freeze, as it rapidly evaporates

2) Ideally tissues are not frozen directly on liquid nitrogen directly, as the vapor between block and liquid N2 may cause some freezing variation. However, without other alternatives, this is an adequate method to freeze cryomolds. **Remember, hold the cryomold in the liquid N2 only up to the top of the cryomold well** – do not submerge the sample! This will result in distortion and damage to the tissue due to boiling of liquid N2 when it comes into contact with the OCT.

Freezing if you have access to isopentane, dry ice, and liquid N2:

Ideally, tissues are frozen in isopentane chilled in liquid N2 in a bucket of dry ice. The dry ice helps reduce the evaporation rate of the liquid N2. Do this in a hood.



1) A relatively large amount of liquid N2 is generally needed, particularly if there are numerous tissues to freeze, as it rapidly evaporates
2) Dry ice (fragmented or pelleted) is placed in a container like an ice bucket or styrofoam container.
3) A second container is used to hold liquid N2 for the freezing process and sits in the dry ice container, surrounded by dry ice. The container holding the liquid N2 must be made of a material that will not crack at cold temperatures.
4) A third container will hold the isopentane on tope of the liquid nitrogen. Often a stainless-steel bowl or container works well to sit in the liquid N2 container.
5) Carefully fill the liquid N2 container with liquid N2.
6) Carefully place the isopentane container with isopentane into the liquid nitrogen (liquid nitrogen will boil – use caution!!) and cover it for a few minutes to allow adequate chilling.
7) **Ensure that the liquid N2 is at an adequate level to uniformly chill the isopentane for the entire time you are freezing tissues.**
8) Freeze samples as previously described (do NOT submerge sample – hold in place until entire sample in well is white and opaque).
9) Place frozen samples on dry ice until they can be stored at -80.

Freezing if you *only* have access to isopentane and liquid N2:

1) A relatively large amount of liquid N2 is generally needed, particularly if there are numerous tissues to freeze, as it rapidly evaporates
2) Fill an appropriate container with liquid nitrogen (e.g., black bucket).
3) Fill a small container with isopentane to be immersed in the liquid N2. Often a stainless-steel bowl or container works well to sit in the liquid N2 container.
4) Carefully immerse cup with the isopentane container into the liquid nitrogen (liquid nitrogen will boil – use caution!!) and cover it for a few minutes to allow adequate chilling. The levels of these two solutions should be the same for even freezing of your specimen.
5) *Keep adding liquid N2 into the container to ensure that the liquid N2 is at an adequate level to uniformly chill the isopentane for the entire time you are freezing tissues.

6) Freeze samples as previously described (do NOT submerge sample – hold in place until entire sample in well is white and opaque).
7) Place frozen samples immediately into the -80 freezer.

Freezing if you *only* have access to isopentane and dry ice:

1) Dry ice (fragmented or pelleted) is placed in a container like an ice bucket or styrofoam container. A slurry may be made by adding isopropyl alcohol to allow for more uniform cooling of the isopentane.
2) Place metal cup or other container into the dry ice/dry ice – alcohol slurry.
3) Fill cup with isopentane and cover the entire bucket to allow isopentane to cool adequately
4) Ensure that the dry ice/ dry ice – alcohol slurry is at least at the same level as the isopentane in the container.
5) Freeze samples as previously described (do NOT submerge sample – hold in place until entire sample in well is white and opaque).
6) Place frozen samples on dry ice until they can be stored at -80.

http://mhpl.facilities.northwestern.edu/files/2013/10/Tissue-Freezing-Presentation-5-26-20111.pdf

Freezing of FIXED tissues

Freezing fixed tissues is performed in the same way as fresh tissue. Freezing fixed tissues may be required for specific stains, such as Oil Red O, for which routine processing will remove the lipid identified by this stain.

Some samples, particularly brain, may be perfused with 4% PFA followed by **sucrose cryoprotection**. Typically the animals are perfused with 4% PFA. The tissues is then immersion fixed in 4% PFA overnight. Samples are then moved to a 15% sucrose in PBS solution until the tissue sinks and then moved to a 30% sucrose in PBS solution until the tissue sinks. The tissue is then ready for freezing.