DISCOVERIES THAT MAKE A DIFFERENCE

Core Imaging Facility

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Preparation of Tissue for Cryoembedding

1. Fix tissue (if applicable). Rinse 2-3 times with buffer.

2. Transfer tissue to 20-30% sucrose. Leave in solution at 4°C overnight, or until tissue no longer floats.

3. Obtain and crush dry ice. Fill the base of a Styrofoam container ~1/2 full with the crushed dry ice.

4. Fill a square staining jar (or other suitable temperature resistant vessel) with 2-methylbutane.

5. Place the container in the dry ice, and surround the sides with additional dry ice, up to the level of the 2-methylbutane.

6. Insert a digital temperature probe to monitor the temperature of the 2-methylbutane. Optimal temperature is -60°C.

7. Add small chunks of dry ice to accelerate the cooling process. (You will notice that when you begin this step, the 2-methylbutane will boil rapidly. As you approach your optimal temperature, this reaction noticeably decreases.)

8. Label your mold with a Sharpie or other lab marker.

9. Partially fill a mold with frozen tissue matrix (We use 1:1 TFM/OCT.)

10. Blot tissue to remove excess solution. Arrange tissue near the bottom center of the mold so the tissue is easily exposed when cut.

11. Cover the remaining exposed tissue with tissue embedding medium, being careful to remove any bubbles near the tissue.

12. Hold base molds with tissue in pre-chilled 2-methylbutane so that the sides of the mold are in contact with the solution, but the block is not submerged. The block will begin to solidify (turn opaque) from the sides to the center. When the entire block is opaque, submerge the block briefly in the 2-methylbutane.

13. Remove tissue block from 2-methylbutane and place frozen blocks on dry ice.

14. Store frozen blocks in a sealed container in the –80°C freezer until they are needed for sectioning.

Preparation of Frozen Tissue for Cryosectioning

1. About 30 minutes prior to sectioning, remove blocks from the –80°C freezer and place in the cryostat to allow them to warm up to cutting temperature.

2. Remove embedded tissue from the plastic mold and adhere to cryostat chuck with a small amount of O.C.T. or other tissue freezing medium. Make sure the side that you adhere to the chuck is not the side closest to the tissue.

3. Position the sample through changes in rotation and angle of the chuck holder until you are getting a full, even section of tissue. Sections for most routine applications are cut at 7 microns and picked up on a glass slide.

4. If the block is to be saved and recut later, place a small amount of frozen tissue matrix into the bottom of the plastic mold, then replace the tissue block into the mold making sure there are no air bubbles over the cut surface of the tissue. Allow the matrix to freeze, then store in –80°C freezer in a sealed, airtight container until ready to use.

Preparation of Frozen Slides for Staining

NOTE: The following steps should only be performed on sections immediately prior to staining.

If post-fixation is required:

1. Fix sections in cold acetone (-20°C) for 2 minutes.
2. Dry fixed slides completely (1 hour at room temp).
3. Rinse 2-3 times in distilled water to remove matrix.
4. Stain accordingly.

If no post-fixation is required:

1. Rinse 2-3 times in distilled water to remove matrix.
2. Stain accordingly.