

Prepare Tissue for Frozen Sections

There are two kind of frozen tissue which are used for immunocytochemistry: Fresh frozen tissue and fixed frozen tissue. The fresh frozen tissue will be frozen immediately after the animal was dissected. The fixed frozen tissue should be fixed first then frozen.

Prepare Tissue for Fresh Frozen Sections

1. Before you dissect the animal get one special container filled with liquid nitrogen and another container filled with some dry ice.
2. Label base mold and partially fill the mold with OCT.
3. Remove desired tissue and place in cold PBS and wash out the blood.
4. Transfer tissue to a clean petri dish and use the kimwipes to absorb the PBS on the tissue surface.
5. Place tissue in pre-labeled base molds filled with OCT. Try to arrange tissue flat in OCT near the bottom so tissue is easily exposed when sections are cut.
6. Use forceps to hold base mold edge and place the base mold into the surface of the liquid nitrogen and just let bottom of base mould to touch the surface of the nitrogen. Hold until the tissue solidifies (around 30 seconds). Note: If block is left in too long, it may crack.
7. Remove tissue from liquid nitrogen and place blocked tissue on dry ice. (Tissue may be kept in plastic container or plastic bags.)
8. Store frozen tissue block in -80°C freezer until sectioning.

Prepare Tissue for Fixed Frozen Sections

1. Remove desired tissue and place in cold PBS and wash out blood.
2. Place tissue into fixative and fix for 2-4 hours or overnight depend on the tissue sizes.
3. Wash with cold PBS 1 min.
4. Wash with PBS 10 or 20 min X3. (During wash steps get one special container fill with liquid nitrogen and another container fill with same dry ice. Label base mold and partially fill the mold with OCT.
5. Transfer tissue to a clean petri dish and use the kimwipes to absorb the PBS on the tissue surface.
6. Place tissue in pre-labeled base molds filled with OCT. Try to arrange tissue flat in OCT near the bottom so tissue is easily exposed when sections are cut.
7. Use forceps to hold base mold edge and place the base mold into the surface of the liquid nitrogen and just let bottom of base mould to touch the surface of the nitrogen. Hold until the tissue solidifies (around 30 seconds). Note: If block is left in too long, it may crack.
8. Remove tissue from liquid nitrogen and place blocked tissue on dry ice. (Tissue may be kept in plastic container or plastic bags.)
9. Store frozen tissue block in -80°C freezer until sectioning.