

Summary of Frozen vs. Formalin Fixed Paraffin Embedded (FFPE) Tissues

Frozen Tissues

Frozen tissues that are correctly frozen (with OCT compound in a dry ice/isopentane slurry) are cryosectioned in a cryostat similar to the one shown in the photograph below.



Frozen sections are best used for immunohistochemistry assays, but morphologic detail is not usually well preserved.

The cabinet is kept cool so that frozen sections (5 to 20 μm in thickness) may be cut at varying temperatures, from -15°C (for solid organs like spleen, liver, kidney, heart) to -30°C (for delicate or difficult to section organs or tissue, like brain or adipose tissue).

Tissues that are FROZEN for histopathologic use must be frozen using specific protocols.

Optimum Cutting Temperature (OCT) Compound (VWR Cat. No. 25608-930) is used to surround and CRYOPROTECT the fresh tissue, which is placed in a vinyl mold with more OCT, and then this is immersed in a bath of dry ice and 2-methylbutane (Fisher Cat. No. 03551-4)

It is important to make sure that the tissue juices have been thoroughly blotted away from around the tissue about to be frozen.

Do not use ethanol with dry ice – ethanol does not freeze!

Oil Red O stains on adipose tissue have to be performed on frozen material. Remember, it is technically very challenging to cut frozen sections of adipose tissue; however, one has to suffer through that in order to do the Oil Red O stains.

If you would like to detect the presence of **GFP** label in the tissues, you **MUST** perfusion fix the animal after sacrifice, before freezing the organs.

After the OCT turns white, the frozen tissue block is snapped out of the mold, placed in labeled plastic bags, and into a box in the -80°C freezer until the need arises for frozen sections. Before freezing in OCT, many investigators additionally place thinly sliced, fresh, paraformaldehyde fixed tissues in 30% sucrose in order to cryoprotect the tissues. In our experience, cryoprotection in sucrose has not been necessary for many of the organs we have analyzed.

If not frozen well, the tissues will shatter during sectioning and will show freeze artifacts. Shattered tissue cannot be used for any immunostaining assays.

Summary: frozen tissues are cryosectioned in a cryostat and frozen sections (at 5, 10, or 20 μm thick) are thaw-mounted onto coated glass slides for use in immunohistochemistry assays using a variety of markers.

Paraffin Sections

Much better morphologic detail is obtained when examining standardly fixed, processed, and stained paraffin sections. It is best to label the cassettes yourself using an indelible marker (***do not use Sharpie, as the ink is soluble in the solvents that are used to process the tissues into paraffin***).

It is also extremely important to confer with the histotechnician when you deliver the sample: this will allow both of you to determine the correct orientation of the tissues so that you will receive properly oriented, embedded, sectioned, and stained slides to review and photograph.

Reminder: fixatives take a **long** time to infiltrate tissue, so the thinner the slices that are placed in the labeled cassette, the better fixed they will be. Well-fixed tissues will have better morphologic detail on the stained sections.

If there is a plan to use the fixed tissue sections for immunohistochemical assays, also make sure you confer with the histotechnician ahead of time. remember ---- do not fix for longer than 24 hours in paraformaldehyde or formalin. Transfer to 70% alcohol and take to the histology lab for immediate processing, in order to be able to detect non-denatured epitopes easily.

A bench top microtome is used to make these paraffin sections, as shown in the photo.

To obtain the best morphologic detail of fixed sections, it is VERY IMPORTANT that fresh tissue is THINLY SLICED before being placed into the fixative solution of choice, so that the fixative can do its job properly.

Fix thin slices of tissue labeled cassettes in 10 volumes of fixative for at least 24 hours and transfer to 70% alcohol for processing into paraffin.