

IMMUNOHISTOCHEMISTRY: FROZEN SECTION VERSUS PARAFFIN SECTION

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Frozen sections are able to preserve the immune activity of antigen which may have been embedded during the tissue processing, thus exhibiting more sensitive reactivity of antigen-antibody binding, also antigen retrieval step can be removed compared to conventional paraffin section. Please note that frozen sections are usually thicker than paraffin slides due to the operation difficulty in block sectioning.

1.

Prepare frozen tissue sections:

- a. Fresh tissue and fixation using 4% PFA in 4°C overnight are recommended.
- b. Wash tissue 3 times with PBS for 5 minutes each.
- c. Immerse tissue in 20–30% sucrose for 16–48 h.
- d. Place the tissue block on to a pre-labeled tissue base mold.
- e. Cover the entire tissue block with cryo-embedding media (e.g. OCT).
- f. Slowly place the base mold containing the tissue block into liquid nitrogen until the entire tissue block is submerged into liquid nitrogen to ensure tissue is frozen completely.
- g. Store the frozen tissue block at -80°C until ready for sectioning.
- h. Transfer the frozen tissue block to a cryotome cryostat (e.g. -20°C) prior to sectioning and allow the temperature of the frozen tissue block to equilibrate to the temperature of the cryotome cryostat.
- i. Section the frozen tissue block into a desired thickness (typically 5–60 µm). Keep the section apparatus including the blade and the blade holder clean by polishing with soft paper tissue.
- j. Place the tissue sections onto glass slides suitable for immunohistochemistry.
- k. Sections can be stored in a sealed slide box at -80°C for later use.

Tip

For fast processing of clinical samples, eliminating the fixation step by directly freezing and embedding with OCT, followed by cutting (6–8 µm thickness), is recommended as it would be time-saving and avoid the increased difficulty of sectioning caused by fixation. As for the temperature in the cryostat for unfixed tissues, please refer to the table below:

Cryostat Temperature For Unfixed Tissues	
Brain, liver and lymph node tissues	-10°C/-15°C
Thyroid, spleen, kidney and muscle tissues	-15°C/-20°C
Tissue containing fat	-25°C
Tissue containing plenty of fat	-30°C