ST. MICHAEL'S
UNITY HEALTH TORONTO

RCF
HISTOLOGY

Methods and Techniques for Frozen Tissue Sections

(modified from IHC world)

Procedure

1. Snap frozen fresh tissues tissues in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, embedded in OCT compound in cryomolds. Store frozen blocks at - 80 ºC.
2. If embedding in OCT, you need **sucrose cryoprotection:**
    a. Fix in 4% PFA
    b. 15% sucrose in PBS until tissue sinks
    c. 30% sucrose in PBS until tissue sinks
    d. Embed in OCT and freeze
3. Cut 4-8 um thick cryostat sections and mount on superfrost plus slides or gelatin coated slides. Store slides at - 80 ºC until needed. The slides can be store at -20 ºC for short term storage (within a few weeks).
4. Before staining, warm slides at room temperature for 30-60 minutes and fix in ice cold acetone or other alternate fixatives for 5-10 minutes. Air dry for 30-60 minutes.
5. Wash in PBS or TBS and proceed to standard staining procedure.

Principal Factors for Good Sectioning of Frozen Specimens

1. The temperature must be correct for the specimen being cut.
2. The microtome must be correctly adjusted and operated.
3. The cutting blade must be sharp and set at the correct angle.
4. The anti-roll plate must be correctly adjusted.

(Last reviewed May 23rd, 2025)

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Recommended Temperatures for Cutting Unfixed Frozen Tissues

Tissue Type	Working Temperature (°C)
Brain	-12
Liver	-14
Lymph Node	-14
Kidney	-16
Spleen	-16
Muscle	-20
Thyroid	-20
Skin	-25
Breast	-25
Breast with Fat	-30 or below
Adipose Tissue	-30 or below
Fixed Tissue	**-12 to -17**

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