

PreAnalytiX Supplementary Protocol

Cryo-embedding tissue specimens fixed and stabilized with the PAXgene[®] Tissue System

This protocol is designed for cryo-embedding tissue specimens fixed and stabilized in either the PAXgene Tissue Container (cat. no. 765112) or the PAXgene Tissue FIX Container (50 ml) (cat. no. 765112).

IMPORTANT: Please read the *PAXgene Tissue Container Product Circular* or the *PAXgene Tissue FIX Container (50 ml) Product Circular*, paying careful attention to the "Safety Information" and "Important Notes" sections, before starting this procedure.

For research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Sucrose (high purity grade; e.g., Merck[®], cat. no. 107687)*
- RNase-free water
- Dry ice
- 2-Methylbutane (Isopentane) (e.g., Sigma-Aldrich[®], cat. no. M32631)* or liquid nitrogen
- Standard or small cryomolds (e.g., Tissue-Tek[®] Cryomold[®] Molds, VWR[®], cat. nos. 25608-916 or 25608-922)*
- Cryo-embedding medium (e.g., FSC22 Frozen Section Compound, Leica[®] Biosystems, cat. no. 3801480)*
- Specimen disc (e.g., Leica Biosystems, cat. no. 14047743739)*
- Adhesion slides (e.g., SuperFrost[®] Plus Slides, VWR, cat. no. 631-0108)*

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Starting material

Starting material for cryo-embedding should be a tissue sample cut to a maximum size of 4 x 15 x 15 mm, placed into a standard tissue cassette and fixed and stabilized in either the PAXgene Tissue Container or the PAXgene Tissue FIX Container (50 ml).

Things to do before starting

- Prepare a 30% sucrose solution [w/v] by dissolving 30 g sucrose (high purity grade) in 100 ml RNase-free water.

Procedure

Preparing tissue specimens

1. **Remove the fixed and stabilized tissue from the tissue cassette.**
2. **As preparation for cryo-embedding, cut the fixed and stabilized tissue to a maximum thickness of 2 mm.**
Note: Larger tissue samples will not harden enough to be sectioned with a cryostat.
Note: Smaller tissue samples or biopsies can be cryo-embedded without cutting.
3. **Transfer the tissue specimen into a suitable vial filled with at least 10 ml 30% sucrose solution.**
4. **For cryo-protection, incubate the tissue specimen in the sucrose solution at 2–8°C until the sample sinks to the bottom of the vial.**
Note: Some tissue types (e.g., lung tissue) do not sink. In such cases, overnight incubation is sufficient.
5. **Snap-freeze the tissue specimen either directly in 2-methylbutane cooled on dry ice followed by embedding into a cryo-embedding medium (figure 1, page 5) or place the tissue into a cryomold filled with a cryo-embedding medium for simultaneous snap-freezing and embedding in liquid nitrogen (figure 2, page 6).**

Direct snap-freezing in 2-methylbutane on dry ice and cryo-embedding (see figure 1, page 5)

1. **Fill a Styrofoam box with dry ice. Place a suitable container filled with 2-methylbutane (isopentane) on the dry ice.**
2. **Pre-cool the 2-methylbutane to approximately –60°C.**
Note: Pre-cooling can be accelerated by throwing small pieces of dry ice into the 2-methylbutane.
3. **Remove the tissue specimen from the sucrose solution and dab on an absorbent sheet to remove excess solution.**

4. **Snap-freeze the tissue specimen by immersing it into the pre-cooled 2-methylbutane.**

Note: For optimal preservation of morphology and biomolecules it is essential to freeze the specimen quickly and uniformly.

Note: Do not submerge the tissue specimen into cryo-embedding medium prior to freezing in 2-methylbutane since complete freezing of the tissue specimen in medium will take too long.

5. **Fill an empty cryomold with cryosolidifiable cryo-embedding medium.**
6. **Immerse the filled cryomold into the pre-cooled 2-methylbutane, such that 2-methylbutane contacts all sides of the mold but does not flood the cryo-embedding medium.**
7. **When the cryo-embedding medium begins to turn white, use forceps to remove the tissue specimen from the 2-methylbutane and submerge it into the middle of the cryo-embedding medium.**

Note: Do not wait until the cryo-embedding medium has completely turned white.
8. **Immediately place a specimen disk (specimen holder) on top of the tissue within the cryo-embedding medium and submerge the cryomold completely into the 2-methylbutane.**

Note: It is essential for the quality of the morphology and nucleic acids to work quickly to avoid that the specimen thaws in the cryo-embedding medium.
9. **Keep the cryomold in the 2-methylbutane until the cryo-embedding medium has completely turned white.**
10. **Remove the PAXgene Tissue fixed, cryo-embedded (PFCE) block of tissue from the cryomold and store at -80°C until sectioning.**

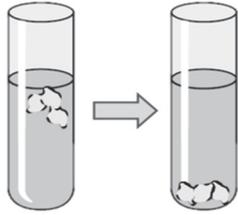
Simultaneous snap-freezing and cryo-embedding using liquid nitrogen (see figure 2, page 6)

1. **Fill an empty cryomold with cryosolidifiable cryo-embedding medium.**
2. **Remove the tissue specimen from the sucrose solution and dab on an absorbent sheet to remove excess solution.**
3. **Place the tissue specimen into the middle of the cryo-embedding medium.**
4. **Place a specimen disk (specimen holder) on top of the tissue within the cryo-embedding medium.**
5. **Using forceps, place the cryomold into liquid nitrogen such that the liquid contacts all sides of the mold but does not flood the cryo-embedding medium.**

Note: Do not hold the cryomold above the liquid level. Freezing in the gas phase takes too long and leads to suboptimal morphology preservation.

Note: Do not submerge the cryomold completely into the liquid nitrogen. Doing so can crack the cryo-block.

6. Allow the cryo-embedding medium to completely turn white, and remove the cryomold from the liquid nitrogen.
7. Remove the PAXgene Tissue fixed, cryo-embedded (PFCE) block of tissue from the cryomold and store at -80°C until sectioning.



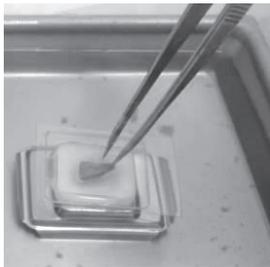
Cut the PAXgene Tissue fixed and stabilized tissue to a maximum thickness of 2 mm. Incubate overnight in 30% [w/v] sucrose.



Snap-freeze the tissue in 2-methylbutane pre-cooled on dry ice.



Fill a cryomold with cryo-embedding medium and pre-cool in 2-methylbutane.



Submerge the frozen tissue into the middle of the cryo-embedding medium.

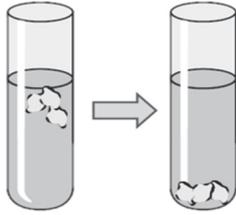


Immediately place a specimen disk on top of the cryo-embedded tissue.

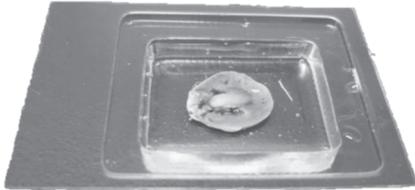


Completely submerge the cryomold with tissue into the 2-methylbutane.

Figure 1: Snap-freezing in 2-methylbutane followed by cryo-embedding.



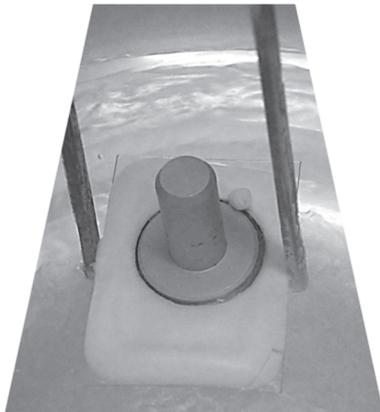
Cut the PAXgene Tissue fixed and stabilized tissue to a maximum thickness of 2 mm. Incubate overnight in 30% [w/v] sucrose.



Fill a cryomold with cryo-embedding medium and submerge the tissue.



Place a specimen disk on top of the tissue.



Using long forceps, hold the cryomold with tissue and specimen disk in liquid nitrogen.

Figure 2: Simultaneous snap-freezing and cryo-embedding using liquid nitrogen.

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