Xenium In Situ for Fresh Frozen Tissues – Tissue Preparation Guide

Introduction

Xenium In Situ for Fresh Frozen Tissues is designed to measure mRNA in tissue sections derived from fresh frozen (FF) and embedded tissue samples and requires a Xenium slide with intact tissue sections as input. Proper tissue handling, storage, and preparation techniques preserve the morphological quality of tissue sections and integrity of mRNA transcripts.

This Tissue Preparation Guide provides guidance on:

- Freezing and embedding tissue samples prior to cryosectioning.
- Best practices for handling tissue samples and Xenium slides before and after cryosectioning.
- Hematoxylin and Eosin (H&E) staining to check tissue quality.
- Cryosectioning of tissue samples and placement of sections on Xenium slides.

Additional Guidance

Refer to the 10x Genomics Support website for additional resources, including How-to Videos. Slides prepared using this guide can be used with:

- Xenium In Situ for Fresh Frozen - Fixation & Permeabilization Demonstrated Protocol (CG000581)
- Xenium In Situ Gene Expression - Probe Hybridization, Ligation & Amplification User Guide (CG000582)

Contents

Xenium In Situ Gene Expression Reagent Kits

Refer to SDS for handling and disposal information

Xenium Slides & Sample Prep Reagents (2 slides, 2 rxns) PN-1000460

Xenium Slides & Sample Prep Reagents
(2 slides, 2 rxns), PN-1000460
Store at –20°C

	#	PN
Xenium Probe Hybridization Buffer	1	2000390
Xenium Post Hybridization Wash Buffer	1	2000395
Xenium Ligation Buffer	1	2000391
Xenium Ligation Enzyme A	1	2000397
Xenium Ligation Enzyme B	1	2000398
Xenium Amplification Mix	1	2000392
Xenium Amplification Enzyme	1	2000399
Reducing Agent B	1	2000087
Xenium Autofluorescence Mix	1	2000753
Xenium FFPE Tissue Enhancer	1	2000798
Xenium Nuclei Staining Buffer	1	2000762
Perm Enzyme B	1	3000553
Xenium Slides (2 pack)	1	3000941

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Only the Xenium Slides (2 pack) are needed for this workflow.

Xenium Slides Kit (2 slides) PN-1000465

Xenium Slides Kit
(2 slides), PN-1000465
Store at –20°C

	#	PN
Xenium Slides (2 pack)	1	3000941

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Purchase the Xenium Slides Kit (2 slides) (PN-1000465) for additional slides as needed.

Recommended Thermal Cyclers

Supplier	Description	Part Number
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module (discontinued)	1851197
Analytik Jena	Biometra TAdvanced 96 SG with 96-well block (silver, 0.2 mL) and gradient function	846-x-070-241 (*where x=2 for 230 V; 4 for 115 V; 5 for 100 V, 50-60 Hz)*
VWR	Gradient thermal cycler, XT^{96} Gradient, with 96-well gradient block and standard lid	76452-153
Marshall Scientific	MJ Research PTC-200 Thermal Cycler	05434-05

Specific Reagents & Consumables

	For Tissue Freezing			
	Item	**Alternatives/Options**	**Vendor**	**Part Number**
☐	Isopentane	Isopentane (2-Methylbutane)	Millipore Sigma	270342
☐	Forceps	Specimen Forceps, Straight, 203 mm (8")	VWR	82027-436
		Specimen Forceps, Straight, 152 mm (6")	VWR	82027-438
	Frozen Tissue Embedding			
☐	Embedding Compound	TissueTek O.C.T. Compound	VWR	25608-930
☐	Embedding Molds	Epredia Peel-A-Way Disposable Embedding Molds	Fisher Scientific	12-20
	Frozen Tissue Sectioning			
☐	Blank Slides *(optional, for sectioning practice)*	Superfrost Plus Microscope Slides	Fisher Scientific	12-550-15
☐	Cryostat	Epredia CryoStar NX70 Cryostat	Fisher Scientific	957020
☐	Brushes	Flat cryostat brush, 10 mm	Fisher Scientific	14-071-00
☐	Specimen Chuck	Thermo Scientific CryoStar NX70 Specimen Chuck	Fisher Scientific	14-071-413
☐	Microtome Blade	MX35 Ultra Microtome Blade, Low Profile	Fisher Scientific	3051835
☐	Slide Mailer	Simport Scientific LockMailer Tamper Evident Slide Mailer	Fisher Scientific	22-038-399
☐	Anti-Roll Plate *(optional)*	Glass Anti-Roll Plate	Fisher Scientific	A78930200
☐	Marker *(optional)*	StatMark Pen	EMS	72109-12
	Additional Materials			
☐	Dry Ice		-	-
☐	Razor Blades		-	-
☐	Polypropylene Beaker			
☐	Ice Bucket or Styrofoam Box		-	-
☐	Aluminum Foil		-	-

For Fixation				
	Item	**Description**	**Vendor**	**Part Number**
☐	PBS	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
☐	Nuclease-free Water	Nuclease-free water (not DEPC-treated)	Thermo Fisher Scientific	AM9932/ AM9937
☐	Formaldehyde	Formaldehyde (37% by Weight/Molecular Biology)	Thermo Fisher Scientific	BP531-500
	or	Formaldehyde Solution	Millipore Sigma	252549, F8775, or 47608
	Paraformaldehyde	Paraformaldehyde 16% Aqueous Solution, EM Grade	Electron Microscopy Sciences	15710
☐	Forceps	Tweezers, 4" Wafer Handling	Excelta Corp	491P-SA-PI
☐	Slide Mailers	Sim port Scientific LockMailer Tamper Evident Slide Mailer	Fisher Scientific	22-038-399
	Additional Materials			
☐	Dry Ice			
☐	Thermal Cycler *(see Recommended Thermal Cyclers)*			
☐	Slide drying rack			
☐	Fume Hood			
☐	Vortex			
☐	Ice bucket			

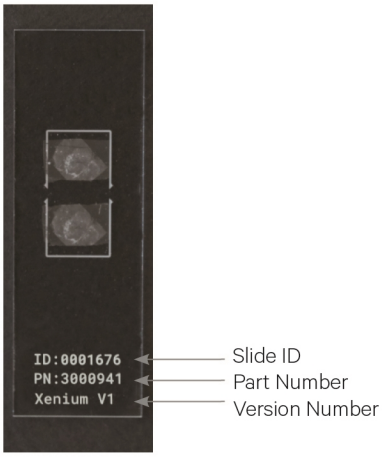
For H&E Staining				
Item		**Description**	**Vendor**	**Part Number**
☐	Hematoxylin	Hematoxylin Solution, Mayer's	Sigma Aldrich	MHS16
☐	Eosin	Eosin Y Solution, Alcoholic	Leica	3801615
☐	Bluing Reagent	Bluing Solution	Dako	CS702
☐	Mounting Media	Surgipath SUB-X Mounting Media (discontinued)	Leica	3801741
		Cytoseal Mountant; 60, 280 and XYL	Fisher Scientific	22-050-262
☐	Ethanol	Ethyl Alcohol, 200 Proof, anhydrous	Millipore Sigma	E7023
		Ethanol absolute ≥99.5%, TechniSolv, pure (Europe)	VWR	83813.360DP

For H&E Staining				
☐	Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
		Xylene, Histological Grade	Millipore Sigma	534056
☐	Forceps	Tweezers, 4' Water Handling	Excelta Corp	491P-SA-PI
☐	Filter Paper	Fisherbrand Qualitative Grade Plain Filter Paper Circles	Fisher Scientific	09-795-H
☐	Coverslips	Fisherbrand Cover Glasses: Rectangles	Fisher Scientific	12-544-EP
		Cover Glasses, Rectangles	VWR	16004-322
	Additional Materials			
☐	Vortex			
☐	Staining jar/dishes			
☐	Wide-bore pipette tips			
☐	Ultrapure/Milli-Q Water *from Milli-Q Integral Ultrapure Water System or equivalent*			

Xenium Slide

- Xenium slides include an imageable area outlined by a white line measuring 12 mm x 24 mm, with an available sample positioning area measuring 235 mm^2 (10.45 mm x 22.45 mm). The available sample positioning area will be referred to as the Sample Area for the remainder of this document.
- The Sample Area is surrounded by fiducials. Tissue sections are placed within the Sample Area without obstructing the fiducials. The imageable area includes the area within the fiducial frame + Sample Area.
- The Sample Area can accommodate as many tissue sections as can fit within the space. Ensure tissue sections (including OCT) DO NOT overlap.
- An etched label denoting the Slide ID, Part, and Version numbers is located at the bottom of the slide. Tissue sections should be placed on labeled-side of slide.

Xenium Slide with Tissue Sections



Tips & Best Practices

Icons

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Tissue Scoring

- OCT block with embedded tissue can be trimmed with a razor blade to fit the Sample Area on the Xenium slide.
- Large tissue samples can be scored during sectioning to generate smaller samples to fit the Sample Area.
- Scoring can be done by making a shallow incision (~1 mm deep) on the cutting surface of the tissue with a razor blade.
- The incision should be shallow. A deep incision may lead to tissue damage and disintegration.
- Once a tissue has been scored, use extra care during sectioning and section handling.

Cryosectioning Temperature

- Cryosectioning temperatures impact tissue section integrity. A temperature setting of −20°C for blade and −10°C for the specimen head is recommended.
- The temperature settings depend upon the local conditions, tissue types, and the cryostat used and should be optimized based on the quality of resulting tissue sections.
- During prolonged sectioning periods, allow the cryostat temperature to equilibrate by briefly closing the chamber.

Sectioning Speed

- Sectioning speed is dependent and impacted by the desired thickness of the sections and the condition of the tissue. Harder and thicker sections require slow sectioning speed.
- Faster sectioning speed may lead to cracks or tears in the sections or damage to the tissue block or cryostat.

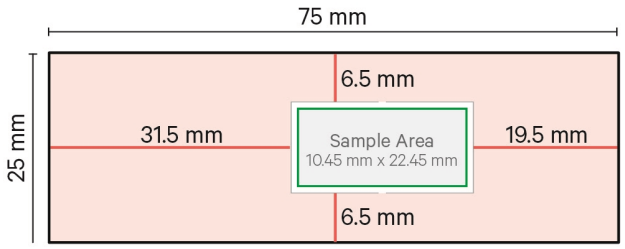
Section Thickness

- Recommended section thickness is 10 μm.

Xenium Slide Template

- Use the following diagram to verify that freshly placed tissue sections are compatible with the Xenium Slide. Reference the image below to draw the sample area on the back of blank slides.

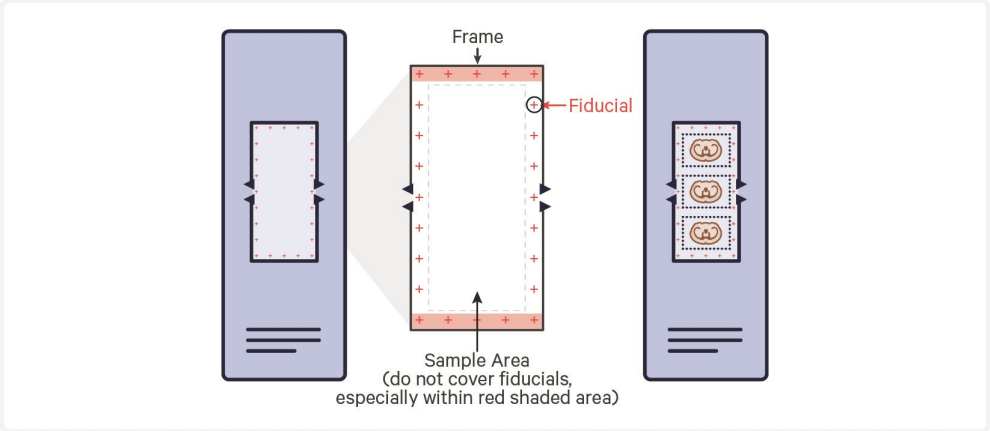
Images are to scale if scaling settings are not modified (select "actual size" or "100%" to print to scale).



- Practice correct section placement within the representative frames using non-experimental blocks.

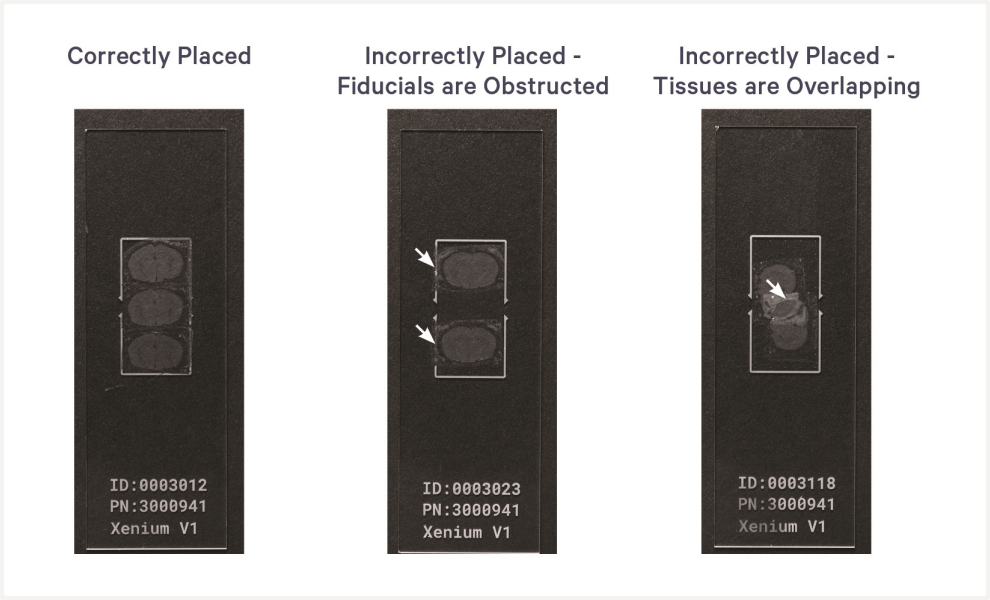
Section Placement on Xenium Slides

- Discard the first couple of sections after facing the block before placing sections on Xenium slides.
- Place the tissue section within the Sample Area on Xenium slides. Avoid covering the fiducials with tissue.



- To assist in section placement, trace the Sample Area on the back of the slide using the provided template in Xenium Slide Template on the previous page.
- The section on the slides should be uniform without any cracks, tears, or folds.
- The OCT surrounding each tissue section should not overlap other sections.

- Once sections are placed on Xenium slides, they cannot be repositioned as this would compromise slide integrity and assay performance.



Tissue Detachment on Xenium Slides

- Monitor section adhesion on Xenium slides throughout the workflow.
- Tissue detachment during the workflow can negatively impact performance. If observed, contact support@10xgenomics.com.
- For more information, refer to Troubleshooting.

Handling Xenium Slides

Handling Xenium Slides Without Tissue Sections

- Store packaged slides at **−20°C**.
- DO NOT touch the surface of the slide.
- Prior to sectioning, slides and slide mailers should be equilibrated to cryostat temperature for:
  - **10 min** if taken immediately from **−20°C**.
  - At least **30 min** if stored at room temperature.
- Once opened, the slide can remain at room temperature in a desiccator for up to one week.

Handling Xenium Slides Containing Tissue Sections

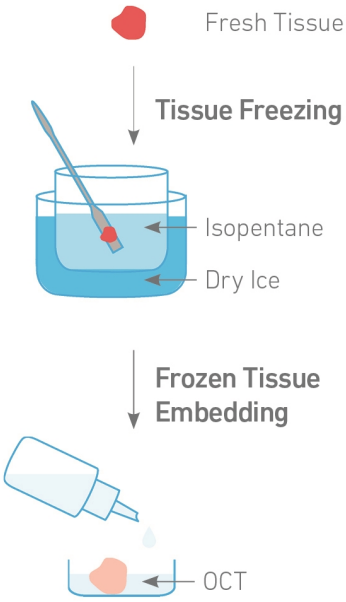
- Slides containing tissue sections are stored in a slide mailer at **−80°C** for up to **4 weeks** after sectioning.

1. Tissue Freezing and Embedding

Overview

This section provides guidance on tissue freezing and embedding. Freshly obtained tissue samples must be snap frozen to prevent RNA degradation and avoid crystal formation, which can lead to morphological damage to the tissue. Once frozen, tissue samples are embedded in a freezing and embedding compound, Optimal Cutting Temperature (OCT), to preserve the structure of the tissue and to provide structural support during cryosectioning.

Alternatively, perform simultaneous freezing and embedding in OCT for tissues with crevices/gaps or tissues that have a tendency to curl.



Tissue Freezing

A bath of isopentane on dry ice is used to freeze the freshly obtained tissue.

Tissue should not be placed directly in liquid nitrogen as the temperature difference may cause boiling on the surface of the tissues, leading to air pockets and uneven freezing. This may crack and morphologically damage the tissue.

Frozen Tissue Embedding

Prior to cryosectioning, frozen tissue samples are embedded in OCT. Unlike paraffin or resin-based embedding techniques, OCT does not interact with proteins and other molecules that impact antigenicity.

OCT embedding of the tissue offers the following advantages:

- Preserves the structure of the tissue and provides structural support during cryosectioning.
- Maintains an optimal temperature during sectioning, thus leading to smooth sections.

1.0 Simultaneous Tissue Freezing & Embedding

Items		Preparation & Handling
Prepare		
☐	Isopentane and dry ice	Fill a polypropylene beaker with ~**200 ml** of isopentane and place in a styrofoam box with dry ice pellets. Let sit for ≥ **30 min.**
☐	OCT	OCT should be at room temperature.
☐	Tissue	Using a rolled up laboratory wipe, absorb excess moisture from the surface of the tissue to limit ice crystal formation.

a. Label the cryomold to mark the orientation of the tissue.

b. Add a few drops of room temperature OCT into the crymold.

c. Using forceps, transfer tissue into the cryomold with OCT. If necessary, adjust tissue orientation and add additional OCT to ensure tissue is fully covered. Confirm there are no bubbles, especially near the tissue. Let sit for **20 sec**.

d. Using forceps, lower the cryomold containing embedded tissue into the isopentane without fully submerging. Keep cryomold in contact with isopentane until the OCT has solidified and turned white.

e. Once frozen, place the cryomold on a piece of aluminum foil on dry ice for **30 min**.

STOP **f.** Store frozen embedded tissue in an airtight sealed container at **–80°C** for **long- term** storage or immediately proceed to Cryosectioning and Section Placement.

Failure to use a sealed container for storage may dehydrate and damage the tissue.

1.1 Separate Tissue Freezing

Items		Preparation & Handling
Prepare		
☐	Isopentane and dry ice	Fill a polypropylene beaker with ~**200 ml** of isopentane and place in a styrofoam box with dry ice pellets. Let sit for ≥ **30 min.**
☐	Tissue	Using a rolled up laboratory wipe, absorb excess moisture from the surface of the tissue to limit ice crystal formation.

a. Using forceps, transfer the tissue onto a piece of aluminum foil.

b. Wrap the tissue in aluminum foil, ensuring the tissue is completely wrapped.

c. Using forceps, lower the wrapped tissue into the isopentane.

- Freezing time is dependent on tissue size and composition. For a 10 x 10 mm tissue, freezing time is ~**1 min**.
- Avoid long freezing times as they can lead to morphological changes.

d. Once frozen, use chilled forceps to lift the wrapped tissue out of the isopentane and placed on dry ice

e. Use chilled forceps to unwrap the foil and transfer tissue to a chilled and airtight sealed container or resealable bag.

f. Store frozen tissue at **–80°C** for **long-term** storage or immediately proceed to the next step (Frozen Tissue Embedding).

To prevent evaporation and dehydration of the tissue sample, snap-frozen tissue sample must be stored in a sealed container.

1.2 Frozen Tissue Embedding

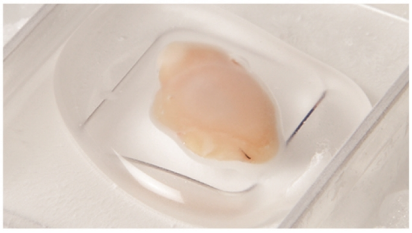
Prepare	
Item	**Preparation & Handling**
Chilled OCT	Place OCT on ice for ≥ **30 min**
Chilled forceps	Chill forceps on dry ice for ≥ **30 min**
Chilled beaker	In a fume hood, pour **200 ml** isopentane into a polypropylene beaker and place the beaker on dry ice for ≥ **30 min**

Confirm	
Item	**Preparation & Handling**
Cryomold	The crymold used for embedding should be of appropriate size to fit the tissue sample.

a. Label an appropriately sized cryomold to mark the orientation of the tissue.

Label the cryomold before adding OCT and tissue. The OCT will quickly turn white once frozen, making it hard to determine tissue orientation later.

OCT Before Freezing



Frozen OCT

b. Fill the cryomold with chilled OCT without introducing bubbles.

c. Place the cryomold on a piece of aluminum foil on dry ice.

d. Using chilled forceps, place the frozen tissue into the OCT, covering any exposed surfaces with additional OCT and noting the tissue orientation. Confirm there are no bubbles, especially near the tissue.

e. Using chilled forceps, immediately transfer the cryomold containing tissue and OCT to the isopentane for **1 min**.

f. Transfer cryomold onto a piece of aluminum foil on dry ice for **30 min**.

g. Store the OCT embedded tissue block in an airtight sealed container at **–80°C** for **long-term** storage or immediately proceed to Cryosectioning & Section Placement.

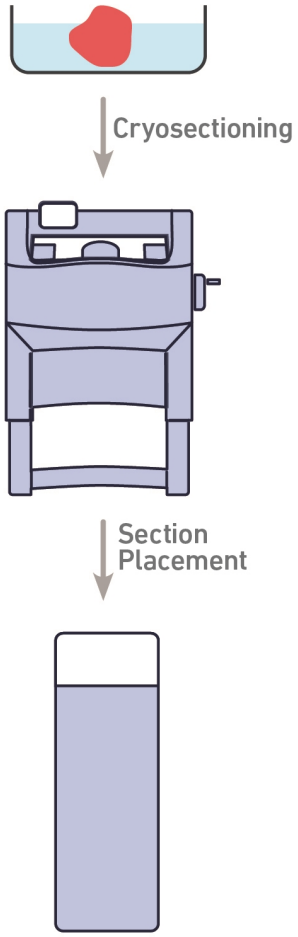
Failure to use a sealed container for storage may dehydrate and damage the tissue.

2. Cryosectioning & Quality Check

Overview

This section provides guidance on cryosectioning of the OCT embedded tissue and placement of a tissue section onto a blank slide for H&E Staining.

Xenium slides are not used for the quality check. Sectioning and placement on Xenium slides for the full workflow is described in step 4.



Cryosectioning

OCT embedded tissue blocks are removed from the **–80°C** storage and cryosectioned in a cryostat to generate appropriately sized sections for blank slides while keeping the samples frozen.

Cryostat Chamber Specifications

This protocol describes the use of a Cryostar NX70 Cryostat with specific capabilities. Alternatively, use a different cryostat with the following features.

Function	Notes
Main Cryochamber	Maintains stable temperatures from –10°C to –20°C
Cryostat Blade	Separate and adjustable temperature control Maintains stable temperatures from –35°C to –5°C
Specimen Head	Separate and adjustable temperature control Maintains stable temperatures from –50°C to +10°C X-axis and Y-axis adjustment
Blade Holder Base	Adjustable cutting angle Adjustable blade position
Cryobar	Rapid cooling

Section Placement & Quality Check

The tissue section is placed on a blank slide.

Fixation & H&E Staining

Tissue sections are fixed then stained in Hematoxylin and Eosin staining solutions. Perform H&E staining according to any preferred protocol. A fixation and H&E protocol are provided in the Appendix. Stained tissue sections are then coverslipped and ready to image. Proceed immediately to imaging following the H&E Staining workflow. Review the H&E image thoroughly to assess tissue quality. Inspect tissue morphology for tissue processing and sectioning artifacts that may contribute to poor assay performance. Refer to 2.3 Fixation & H&E Guidance on page 28 and Troubleshooting on page 38 for more guidance. If quality is satisfactory, proceed with 3. Cryosectioning & Practicing Section Placement on page 29 if additional sectioning practice is needed or proceed to 4.2 Section Placement on Xenium slides on page 36.

2.0 Get Started

Item		10x PN	Preparation & Handling	Storage
Obtain				
☐	Blank Slides	-	Slides should be chilled to cryostat temperature for **≥30 min**. Warm slides will lead to quick melting of the sections.	Ambient
☐	Slide Mailer	-	Slide mailer should be chilled to cryostat temperature for **≥30 min**.	Ambient
☐	OCT-embeded Tissue Block	-	OCT embedded tissue block stored at **–80°C** must be equilibrated to cryostat chamber temperature for **30 min** before sectioning. If the tissue block is too cold, it will lead to section cracking. If the tissue block is too warm, it will lead to section compression or crumpling.	–80°C
☐	Specimen Chuck	-	Specimen chuck should be chilled to cryostat temperature for **≥5 min**.	Ambient
☐	Cryostat	-	Turn cryostat on to chill chamber. Recommended sectioning temperature is **–20°C** for cryostat blade and **–10°C** for the specimen head. Follow manufacturer's manual for detailed operations.	Ambient
☐	Razor Blade *(optional, if scoring)*	-	Chill to cryostat temperature.	Ambient
☐	Anti-roll Plate *(optional)*	-	Anti-roll plate prevents rolling of tissue sections. Chill to cryostat temperature for **≥30 min**. Warm anti-roll plate will damage tissue during sectioning. Optimize the position of anti-roll plate based on the tissue block size. If possible, adjust the position of anti-roll plate before reaching area of interest.	Ambient

2.1 Cryosectioning

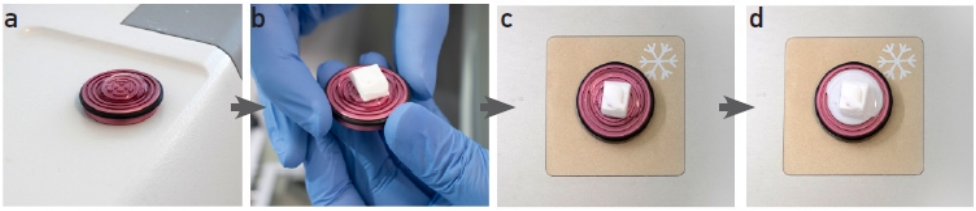
Mount OCT Embedded Tissue Block on the Specimen Stage

a. Fill the specimen stage (chuck) with OCT.

b. Place the OCT embedded tissue block on the stage with the cutting surface facing away from the stage

c. Place the stage and the tissue block on the cryobar inside the cryostat chamber.

d. Allow the OCT and the tissue block to freeze and adhere to the specimen stage for **5 min**.



Remove Excess OCT by Cryosectioning

a. Once frozen, install the stage with the tissue block on to the specimen head of the cryostat and start sectioning to remove excess OCT.

b. Sectioning conditions vary across different tissues and cryostats. Follow manufacturer's recommendation for cryosectioning.

c. Continue sectioning until the tissue is visible.

Tissue Scoring

- Large tissue samples can be scored during sectioning to generate smaller samples to fit the Sample Area. To score, make a shallow incision (~1 mm deep) on the cutting surface of the tissue with a pre-cooled razor blade. The incision should be shallow. A deep incision may lead to tissue damage and disintegration. Once the tissue has been scored, extra care must be taken during sectioning and section handling.
- Example: To examine a specific region within one hemisphere of the mouse brain, scoring can be done by making a ~1 mm shallow incision at the midline of the brain.

Tissue Scoring

2.2 Tissue Sectioning

Confirm	
Item	**Preparation & Handling**
Section thickness setting	Recommended section thickness is **10 µm**.
Anti-roll plate is in place (*optional*)	Anti-roll plate prevents rolling of tissue sections. Chill to cryostat temperature for **≥30 min**. Warm anti-roll plate will damage tissue during sectioning. Optimize the position of anti-roll plate based on the tissue block size. If possible, adjust the position of anti-roll plate before reaching area of interest.
Specimen head temperature	Confirm the temperature of the specimen head. If the sections appear cracked, the specimen head is too cold. If the sections appear crumpled, the specimen head is too warm. Adjust temperature accordingly.

Position of Anti-roll Plate

a. Acquire a tissue section and carefully flatten it out by gently touching the surrounding OCT with cryostat brushes.

If re-sectioning a used tissue block:

- For tissues prone to freeze/thaw damage, such as mouse brain, cut and discard ~7 sections with thickness set to 10 µm prior to collecting first section.
- For other tissues, cut and discard ~5 sections with thickness set to 10 µm prior to collecting first section.

b. Adhere tissue section to slide by gently touching the tissue section to the blank slide.

c. Immediately place a finger on the backside of the slide where the tissue section was placed to allow section to adhere to the slide.

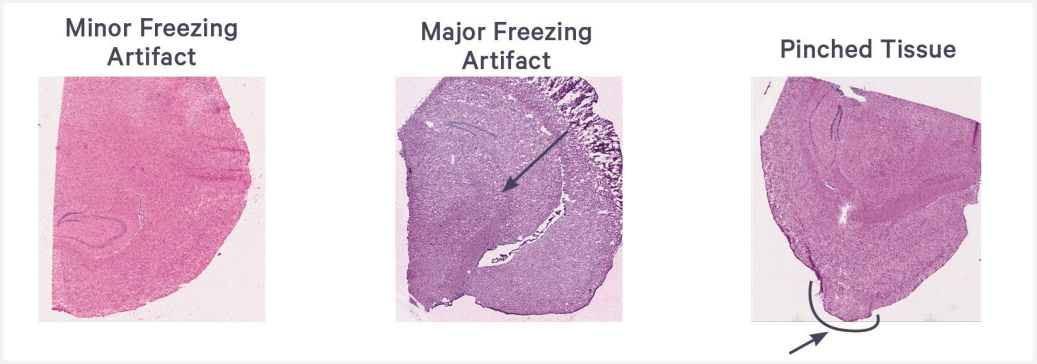
d. Apply OCT to the exposed surface of the block for storage.

e. Proceed to H&E Staining.

2.3 Fixation & H&E Guidance

A fixation protocol is provided in the Appendix. Perform H&E staining according to any preferred protocol. An H&E protocol is provided in the Appendix. Review the H&E image thoroughly to assess tissue quality and select area(s) of interest. If scoring the block is necessary, refer to 2.1 Cryosectioning on page 25.

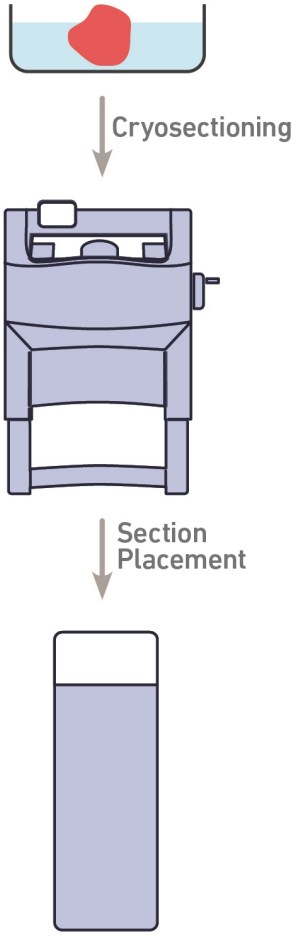
Ensure that the tissue section is free from tissue processing and sectioning artifacts as shown in the images below and in Troubleshooting on page 38. Tissue processing artifacts may include improper snap freezing, squeeze/crush artifacts, ice crystal artifacts, and hemorrhaging. If imaging reveals satisfactory tissue morphology, proceed with 3. Cryosectioning & Practicing Section Placement on page 29 if additional sectioning practice is needed or 4.2 Section Placement on Xenium slides on page 36 for placement of tissue sections on Xenium slides.



3. Cryosectioning & Practicing Section Placement

Overview

After examining the H&E tissue section, practice sectioning the block onto blank slides in preparation for the section placement on the Xenium slide. This practice step is optional.



Cryosectioning

OCT embedded tissue blocks are removed from the **–80°C** storage and cryosectioned in a cryostat to generate appropriately sized sections for slides while keeping the samples frozen. Perform cryosectioning as described in step 2.

Section Placement

Draw the template shown in Xenium Slide Template on page 12 onto a blank slide to simulate the Sample Area on the Xenium slide. The Sample Area can accommodate two tissue sections of 1 cm^2 each, or more if the sections are smaller. Perform section placement as described in step 2.

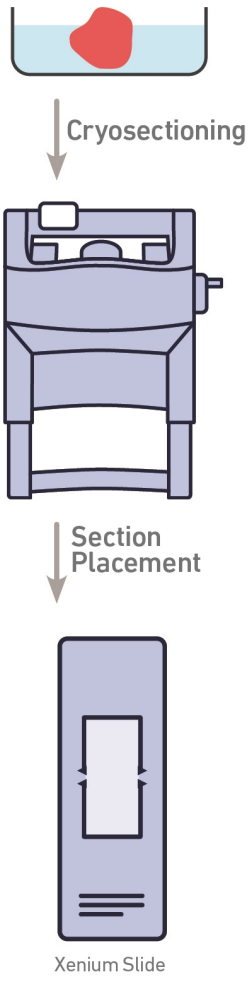
3.0 Get Started

Item		10x PN	Preparation & Handling	Storage
Obtain				
☐	Blank Slides	-	Slides should be chilled to cryostat temperature for **≥30 min**. Warm slides will lead to quick melting of the sections.	Ambient
☐	Slide Mailer	-	Slide mailer should be chilled to cryostat temperature for **≥30 min**.	Ambient
☐	OCT-embeded Tissue Block	-	OCT embedded tissue block stored at **–80°C** must be equilibrated to cryostat chamber temperature for **30 min** before sectioning. If the tissue block is too cold, it will lead to section cracking. If the tissue block is too warm, it will lead to section compression or crumpling.	–80°C
☐	Specimen Chuck	-	Specimen chuck should be chilled to cryostat temperature for **≥5 min**.	Ambient
☐	Cryostat	-	Turn cryostat on to chill chamber. Recommended sectioning temperature is **–20°C** for cryostat blade and **–10°C** for the specimen head. Follow manufacturer's manual for detailed operations.	Ambient
☐	Razor Blade *(optional, if scoring)*	-	Chill to cryostat temperature.	Ambient
☐	Anti-roll Plate *(optional)*	-	Anti-roll plate prevents rolling of tissue sections. Chill to cryostat temperature for **≥30 min**. Warm anti-roll plate will damage tissue during sectioning. Optimize the position of anti-roll plate based on the tissue block size. If possible, adjust the position of anti-roll plate before reaching area of interest.	Ambient

4. Cryosectioning & Section Placement

Overview

This section provides guidance the placement of tissue sections on Xenium slides.



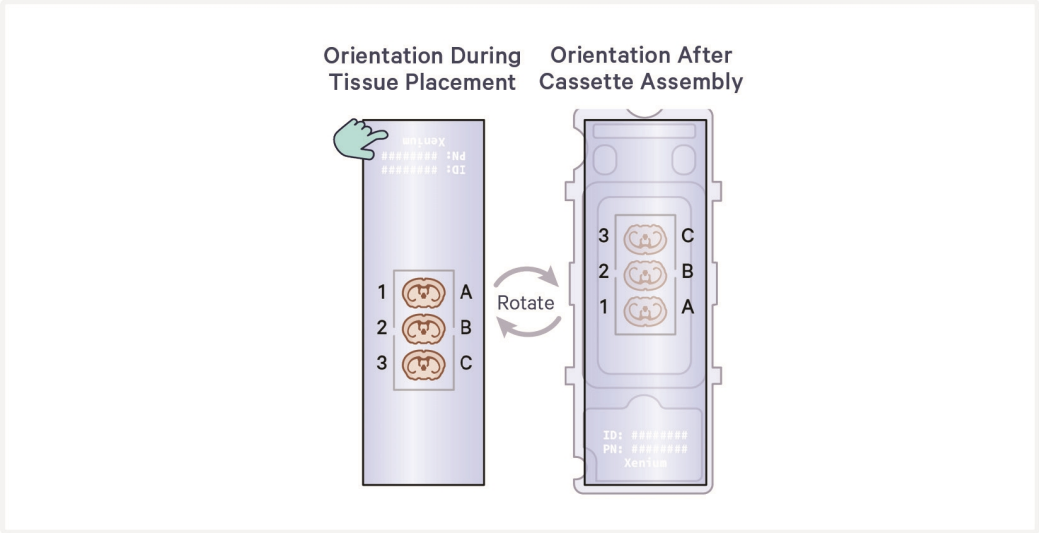
Cryosectioning

OCT embedded tissue blocks are removed from the **–80°C** storage and cryosectioned in a cryostat to generate appropriately sized sections for Xenium slides while keeping the samples frozen. Section as described in step 2.

Section Placement

Prior to section placement, a morphology check is performed to confirm that the sections are free of artifacts. Sections are placed within the frames of the Sample Area on the Xenium slide. The Sample Area can accommodate two tissue sections of 1 cm^2 each, or more if the sections are smaller.

Slides are assembled into the Xenium Cassette with the etched label oriented towards the bottom. If holding the Xenium slide by the etched label for section placement, the slide will be rotated during cassette assembly and placement on the Xenium Analyzer. This results in an image that is rotated compared to initial section placement (refer to image below).

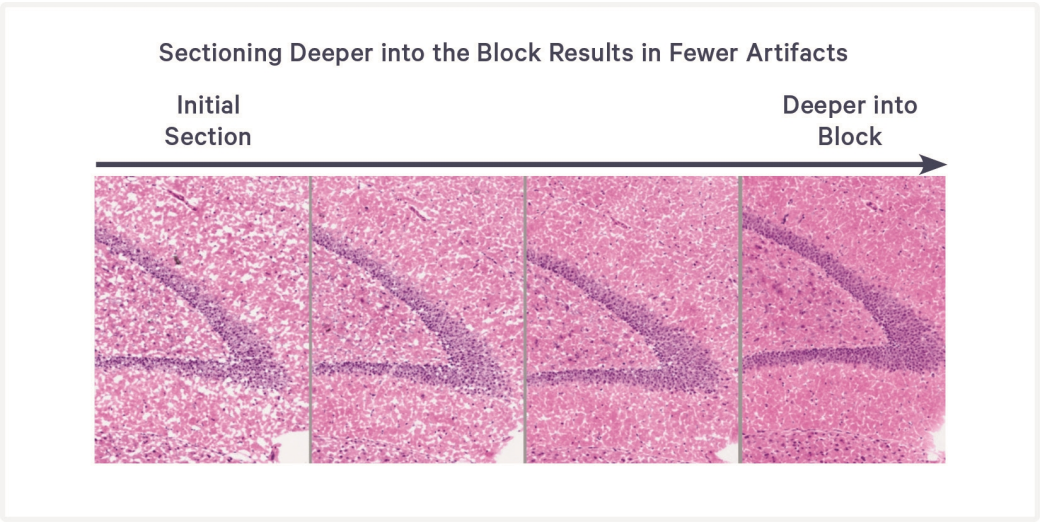


4.0 Get Started

Item		10x PN	Preparation & Handling	Storage
Obtain				
☐	Xenium Slide	-	Slides should be chilled to cryostat temperature for **≥30 min**. Warm slides will lead to quick melting of the sections.	–20°C
☐	Blank Slide	-	Slides should be chilled to cryostat temperature for **≥30 min**. Warm slides will lead to quick melting of the sections.	Ambient
☐	Slide Mailer	-	Slide mailer should be chilled to cryostat temperature for **≥30 min**.	Ambient
☐	OCT-embeded Tissue Block	-	OCT embedded tissue block stored at **–80°C** must be equilibrated to cryostat chamber temperature for **30 min** before sectioning. If the tissue block is too cold, it will lead to section cracking. If the tissue block is too warm, it will lead to section compression or crumpling.	–80°C
☐	Specimen Chuck	-	Specimen chuck should be chilled to cryostat temperature for **≥5 min**.	Ambient
☐	Cryostat	-	Turn cryostat on to chill chamber. Recommended sectioning temperature is **–20°C** for cryostat blade and **–10°C** for the specimen head. Follow manufacturer's manual for detailed operations.	Ambient
☐	Razor Blade *(optional, if scoring)*	-	Chill to cryostat tempreature.	Ambient
☐	Anti-roll Plate *(optional)*	-	Anti-roll plate prevents rolling of tissue sections. Chill to cryostat temperature for **≥30 min**. Warm anti-roll plate will damage tissue during sectioning. Optimize the position of anti-roll plate based on the tissue block size. If possible, adjust the position of anti-roll plate before reaching area of interest.	Ambient
☐	Hematoxylin	-	-	Ambient

4.1 Morphology Check

- Repeated sectioning of tissue blocks may result in damage due to freezing artifacts. Prior to sectioning onto Xenium slides, tissue morphology should be checked for any undesired artifacts. This morphology check will determine if the block needs to be sectioned deeper to avoid artifacts.
- In the example below, the first section removed from the block displays "swiss cheese" morphology, with small spaces between each cell. Subsequent sections show fewer artifacts and are better sections for downstream analysis.



a. Acquire a tissue section as described in step 2 on a blank slide.

b. Remove slide from the cryostat and immerse in a slide mailer filled with hematoxylin for **30 sec**.

c. Immerse in a slide mailer filled with water for **20 sec**.

d. Wipe the back of the slide with a laboratory wipe.

e. Inspect tissue morphology under a microscope.

- If morphology is ideal, cut and discard 3 sections with the section thickness set to 10 µm. Proceed to step 4.2 Section placement on Xenium slides.
- If morphology is not ideal, repeat quality check with additional trimming of the block as needed.

4.2 Section Placement on Xenium slides

Confirm	
Item	**Preparation & Handling**
Section thickness setting	Recommended section thickness is **10 µm**.
Anti-roll plate is in place (optional)	Anti-roll plate prevents rolling of tissue sections. Chill to cryostat temperature for **≥30 min**. Warm anti-roll plate will damage tissue during sectioning. Optimize the position of anti-roll plate based on the tissue block size. If possible, adjust the position of anti-roll plate before reaching area of interest.
Specimen head temperature	Confirm the temperature of the specimen head. If the sections appear cracked, the specimen head is too cold. If the sections appear crumpled, the specimen head is too warm. Adjust temperature accordingly.

Position of Anti-roll Plate

Optional: Before placing sections on Xenium slides, trace the Sample Area onto the back of the slide with a marker. Using an unsupported marker may compromise assay performance. See Xenium Slide Template on page 12 for Xenium slide layout.

a. Once desired tissue section is obtained, carefully flatten it out by gently touching the surrounding OCT with cryostat brushes.

b. Place the section within the Sample Area on the pre-equilibrated Xenium slide by gently touching the section with the active surface of the slide.

DO NOT place sections on a room temperature slide. Slide should be equilibrated to cryostat chamber.

c. Immediately place a finger on the backside of the Sample Area on the slide for a few seconds to allow the section to adhere to the slide.

Ensure that the entire tissue section is fully adhered to the slide and the slide is inside the cryostat chamber throughout section placement. DO NOT remove the slide from the cryostat chamber at any point during

sectioning and tissue placement.

d. Immediately place the slide with tissue section on the cryobar to freeze the section. Continue transferring sections onto the Sample Area.

e. Transfer the slide containing tissue sections to a slide mailer pre-equilibrated in the cryostat.

f. Store slides at **–80°C** for up to **4 weeks** or immediately proceed to Xenium protocols.

- Store up to two slides in a sealed container. Ensure slides are separated by a space to prevent damage. If using an unsealed slide mailer, store in a secondary sealed container, such as a resealable bag.

- Maintain slides containing sections in a cold and low moisture environment. DO NOT expose slides to room temperature as the resulting condensation will cause tissue disintegration.

See Handling Xenium Slides for information on slide handling.

Slide Shipping

- If needed, slides containing tissue sections can be shipped. See Appendix for detailed Shipping Guidelines.

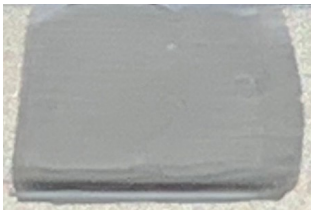
Leftover Tissue Block Storage

- Remove leftover tissue block attached to the specimen stage from the cryostat's specimen head and place onto cryobar.
- Cover the exposed tissue with a thin layer of chilled OCT and allow to freeze.
- To separate the frozen tissue block from the stage, lift the tissue block and the stage from the cryobar and lightly warm the stage with hands or an aluminum block at room temperature

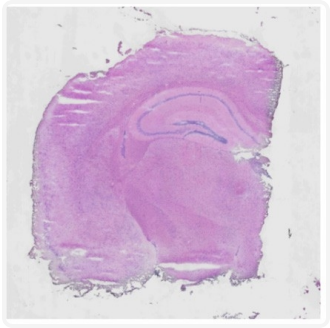
  DO NOT let the block and tissue to fully melt, as this will severely damage the tissue. Separation of the tissue block from the specimen stage is optional. The frozen tissue block can be stored attached to the specimen stage in a sealed container at –80°C.
- Immediately place the tissue block in dry ice. Ensure that the melted areas have refrozen.
- Store in a sealed container at **–80°C** for **long-term** storage.

Troubleshooting

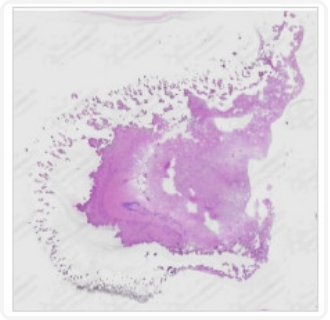
Impact of Cryostat Specimen Head Temperatures on Tissue Tearing

-10°C	-14°C	-20°C	-30°C
			
Normal Section			Tissue section has significant tearing.

Impact of Condensation on Tissue Sections



No Condensation. Intact tissue section.



Tissue degraded due to condensation. DO NOT leave slides at room temperature, especially with fresh sections as the resulting condensation will cause tissue disintegration.

Appendix

Fixation and H&E Staining

Tissue sections should be fixed and H&E stained to assess quality.

Fixation Items		10x PN	Preparation & Handling	Storage
Obtain				
☐	Nuclease-free Water	-	-	Ambient
☐	10X PBS	-	-	Ambient
☐	Formaldehyde or Paraformaldehyde	-	-	Ambient
☐	Slide Mailers	-	-	Ambient
☐	Forceps	-	-	Ambient

H&E Staining Items		10x PN	Preparation & Handling	Storage
Obtain				
☐	Hematoxylin	-	-	Ambient
☐	Eosin	-	-	Ambient
☐	Bluing Reagent	-	-	Ambient
☐	Mounting Media	-	-	Ambient
☐	Xylene	-	-	Ambient
☐	Ethanol	-	Prepare Ethanol dilutions using Milli-Q water.	Ambient
☐	Forceps	-	-	Ambient
☐	Coplin Jars/Staining Dishes	-	-	Ambient
☐	Milli-Q Water	-	-	Ambient

Preparation - Buffers

Prepare all buffers fresh according to the tables below before retrieving tissue sections from **-80°C.**

TIPS *Prepare buffers in appropriate sized conical tube or bottle and transfer carefully to corresponding slide mailer.*

a. Prepare 1X PBS. Add reagents in the order listed. Invert gently to mix. Maintain at room temperature. This volume of 1X PBS is sufficient for washes in all subsequent steps of this Demonstrated Protocol.

1X PBS				
Items		**Stock**	**Final**	**Total Amount (ml)**
☐	Nuclease-free water	-	-	9
☐	RNase free PBS	10X	1X	1
☐	**Total**	**-**	**-**	**10**

b. Using 1X PBS from step 1.1a, prepare Fixation Solution using either Formaldehyde or Paraformaldehyde according to the appropriate table below. Add reagents in the order listed. Invert gently to mix. Maintain at room temperature.

Fixation Solution *(using Formaldehyde)*				
Items		**Stock**	**Final**	**Total Amount (ml)**
☐	1X PBS	-	-	9.0
☐	Formaldehyde	37%	3.7%	1.0
☐	**Total**	**-**	**-**	**10.0**

OR

Fixation Solution *(using Paraformaldehyde)*				
Items		**Stock**	**Final**	**Total Amount (ml)**
☐	1X PBS	-	-	7.5
☐	Paraformaldehyde	16%	4%	2.5
☐	**Total**	**-**	**-**	**10.0**

c. Dispense 10 ml of Fixation Solution into one slide mailer.

Slide Preparation

a. Place Xenium Thermocycler Adaptor in thermal cycler set to incubate at **37°C.** DO NOT close the lid.

Ready the Fixation Solution Mailer and a timer set to 1 min, which are needed in the following steps.

b. Prepare an ice bucket of dry ice.

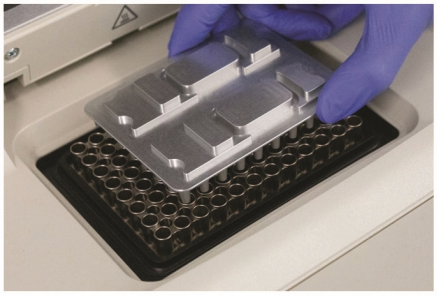
c. Remove slide mailer containing stored fresh frozen tissue slide(s) from **-80°C** and bury into the dry ice.

TIPS

Alternatively, submerge an uncapped empty slide mailer in dry ice and incubate for **5 min**. *Remove slides from* **-80°C** *storage with a pair of forceps and immediately place in pre-chilled empty slide mailer on dry ice.*

d. Using a pair of slide forceps, move the slide(s) from dry ice to the **37°C** pre-heated thermal cycler for **1 min**. Place slide on the Thermocycler Adaptor with the tissue side facing up. Ensure the Sample Area is aligned with the corresponding area on the Thermocycler Adaptor. DO NOT close the lid.

Incubation in a Thermal Cycler



Place Thermocycler Adaptor

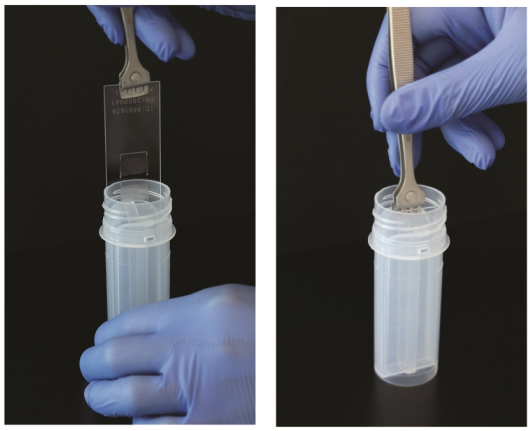
Incubate Slide for 1 min at 37°C

Fixation

a. **Immediately** remove slide from thermal cycler following incubation. Gently immerse slide in the Fixation Solution Mailer using slide forceps and incubate for **30 min** at **room temperature**.

Formaldehyde and Paraformaldehyde should be handled in a biosafety hood due to their hazardous nature. Transfer slides immediately to Fixation Solution following removal from thermal cycler to prevent formation of freezing artifacts on the slides.

Slide Immersion



b. Gently immerse slide in the 1X PBS Mailer 1 and incubate for **1 min** at **room temperature**.

Proceed directly to H&E staining. Leave slide in PBS until H&E reagents have been prepared.

H&E Preparation

TIPS *Prepare buffers in appropriate sized conical tube or bottle and transfer carefully to corresponding coplin jar.*

a. Filter Hematoxylin & Eosin solutions using filter paper before starting H&E Staining protocol.

b. Prepare sixteen total coplin jars for H&E Staining steps.

For H&E Staining		
Items		**Preparation & Handling**
☐	Hematoxylin Solution	Label one coplin jar as Hematoxylin Jar. Fill to capacity with Mayer's Hematoxylin Solution.
☐	Bluing Solution	Label one coplin jar as Bluing Solution Jar. Fill to capacity with Bluing Solution.
☐	70% Ethanol	Label one coplin jar as 70% Ethanol Jar. Fill to capacity with 70% ethanol.
☐	95% Ethanol	Label three coplin jars as 95% Ethanol Jar 1, 2, and 3. Fill to capacity with 95% ethanol.
☐	Eosin Solution	Label one coplin jar as Eosin Solution Jar. Fill to capacity with Eosin Solution.
☐	100% Ethanol	Label two coplin jars as 100% Ethanol Jar 1 and 2. Fill to capacity with 100% Ethanol.
☐	Xylene	Label two coplin jars as Xylene Jar 1 and 2. Fill to capacity with Xylene.
☐	Milli-Q Water	Label five coplin jars as Milli-Q Water Jar 1, 2, 3, 4, and 5. Fill to capacity with Milli-Q Water.

TIPS *Alternatively, a slide staining dish can be used in place of a coplin jar. Adjust volumes accordingly. Use xylene-resistent dishes, gloves, and forceps during workflow.*

H&E Staining

H&E Staining steps should be performed in a fume hood due to the hazardous nature of xylene. Xylene jars should be covered at all times to prevent evaporation.

a. Gently immerse slide in the Milli-Q Water Jar 1 for **2 min** at **room temperature**.

TIPS

Water immersions may be performed in glass beakers containing Milli-Q water, if preferred.

b. Gently immerse slide in the Hematoxylin Solution Jar for **20 min** at **room temperature**.

c. Gently immerse slide in the Milli-Q Water Jar 2 for **1 min** at **room temperature**.

d. Gently immerse slide in the Milli-Q Water Jar 3 for **1 min** at **room temperature**.

e. Gently immerse slide in the Milli-Q Water Jar 4 for **1 min** at **room temperature**.

f. Gently immerse slide in the Bluing Solution Jar for **1 min** at **room temperature**.

g. Gently immerse slide in the Milli-Q Water Jar 5 for **1 min** at **room temperature**.

h. Gently immerse slide in the 70% Ethanol Jar for **3 min** at **room temperature**.

i. Gently immerse slide in the 95% Ethanol Jar 1 for **3 min** at **room temperature**.

j. Gently immerse slide in the Eosin Solution Jar for **2 min** at **room temperature**.

k. Gently immerse slide in the 95% Ethanol Jar 2 for **30 sec** at **room temperature**.

l. Gently immerse slide in the 95% Ethanol Jar 3 for **30 sec** at **room temperature**.

m. Gently immerse slide in the 100% Ethanol Jar 1 for **30 sec** at **room temperature**.

n. Gently immerse slide in the 100% Ethanol Jar 2 for **30 sec** at **room temperature**.

o. Gently immerse slide in the Xylene Jar 1 for **3 min** at **room temperature**.

p. Gently immerse slide in the Xylene Jar 2 for **3 min** at **room temperature**.

Coverslipping

Prior to mounting the coverslip, ensure that the slide is dry. Moisture on the surface of the slide may result in faulty mounting. Wipe away any residual droplets with a lint-free laboratory wipe.

a. Place slide on a flat, clean, non-absorbent work surface.

b. Using a **wide-bore** pipette tip, add **150-200 µl** mounting media to uniformly cover all tissue sections on the slide.

c. Apply the coverslip at an angle on one end of the slide. Slowly lower the coverslip, without introducing bubbles. Allow mounting media to spread and settle.

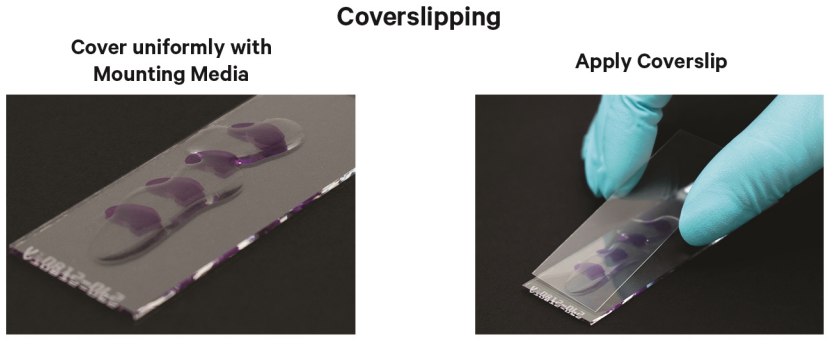
d. If needed, remove any large excess of mounting media by carefully wicking away from the edge of the coverslip with a lint-free laboratory wipe. Be careful not to move coverslip and disturb the tissue.

e. Dry the coverslipped slide for **30 min** at **room temperature**.

STOP

f. Once coverslipping is complete, **immediately** proceed with imaging.

If imaging reveals satisfactory tissue morphology, proceed with 4. Cryosectioning & Section Placement on page 32.



Slides in images are representative

Shipping Guidance

- After sectioning, place up to two slides in a slide mailer.
- Place slide mailer in a sealed bag with a bag of desiccant at **-80°C** until ready for shipment.
- When ready for shipment, place mailer in a styrofoam box with dry ice.

References

1. Xenium In Situ for Fresh Frozen Tissues – Fixation & Permeabilization Demonstrated Protocol (CG000581).

Document Revision Summary

Document Number	CG000579
Title	Xenium In Situ for FF – Tissue Preparation Guide
Revision	Rev C
Revision Date	August 2023
Specific Changes	• Added recommended thermal cycler list for tissue fixation protocol in the Appendix. • Including guidance on precooling the anti-roll plate prior to sectioning. • Corrected Xenium slide storage guidelines in Step 4.

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