Visium CytAssist Spatial Gene Expression for Fresh Frozen – Tissue Preparation Guide

Introduction

The Visium CytAssist Spatial Gene Expression Solution analyzes mRNA from tissue sections on blank slides. Proper tissue handling and preparation techniques preserve the morphological quality of the tissue sections and the integrity of mRNA transcripts. This is critical for downstream library preparation and generation of high quality sequencing data using the Visium CytAssist Spatial Gene Expression protocols.

The Tissue Preparation Guide provides guidance on:

- Best practices for handling tissue samples and blank slides before and after cryosectioning.
- Freezing and embedding tissue samples prior to cryosectioning.
- Cryosectioning of tissue samples and placement of sections on blank slides.
- Assessment of RNA quality.
- Tested blank slides compatible with the Visium CytAssist Spatial assay.

Additional Guidance

This protocol was demonstrated with several tissue types (visit the 10x Genomics support website for a detailed list). Additional optimization may be required for the preparation of specialized tissues, such as tissue with high fat content.

The slides prepared using the Tissue Preparation Guide can be used with:

- Visium CytAssist Spatial Gene Expression for Fresh Frozen – Methanol Fixation, H&E Staining, Imaging & Destaining Demonstrated Protocol (CG000614)
- Visium CytAssist Spatial Gene Expression User Guide (CG000495) Rev C or later

Contents

Tips & Best Practices

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Best Practices

- Best practices for handling any tissues include using sterile techniques, nuclease-free reagents and consumables.

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. Settings 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 depends upon 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 for most tissue types is 10 µm, but tissues from 10–20 µm are compatible with the assay. Tissues with higher fat content (e.g., breast tissue) may require sections closer to 20 µm.

Section Placement on Blank Slides

- After section placement, blank slides are referred to as tissue slides.
- Prior to section placement, draw an outline of the allowable area on the back of the blank slide to ensure downstream compatibility with the Visium CytAssist Tissue Slide Cassette and Visium CytAssist instrument (Refer to Determining Allowable Area). Drawing should be removed after tissue placement.
- If working with multiple sections on multiple tissue slides, ensure that sections are placed in the same location on the tissue slides for improved imaging efficiency.
- If placing multiple sections on a blank slide:
  - Ensure that tissue sections and associated OCT do not overlap with other tissue sections and associated OCT.
  - Ensure sections previously placed on the slide are not thawed while placing other sections.
  - Ensure all sections are placed during the same sectioning event.
  - Only one section from each tissue slide should be used for each Visium CytAssist Spatial Gene Expression Slide Capture Area.

Practice Section Placement

- Practice correct section placement using non-experimental blocks.
- Practicing section placement also allows for determining an ideal tissue thickness for the tissue type or block.
- Sections should be placed on the compatible blank slides listed in the Specific Reagents and Consumables section or similar slides within specified dimensions. For more information, refer to Visium CytAssist Tested Slides.

Handling Tissue Slides

- Keep tissue slides on dry ice.
- If placing in a slide mailer or 50-ml centrifuge tube after sectioning, mailer or tube should be pre-cooled to cryostat temperature for 10–15 min.
    - Immediately place storage container on dry ice for transport to a -80°C freezer for long-term storage.
    - Avoid tissue slides touching one another while in storage.

Determining Allowable Area

Use the following diagrams to verify that freshly placed tissue sections are compatible. Reference the images below to draw the allowable area on the back of blank slides (remove after tissue placement). Images are to scale if scaling settings are not modified. To verify, ensure that the first block in section A measures 6.5 x 6.5 mm.

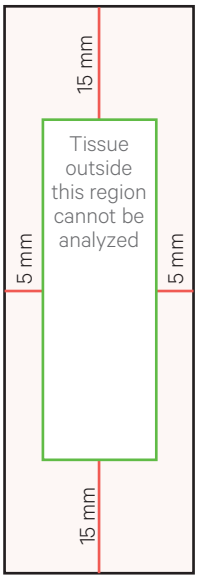
A **Ensure that the area of interest will fit within the Capture Area of a Visium CytAssist Spatial Gene Expression Slide.**

Tissue sections larger than these Capture Areas may be placed on the slide, but only the tissue within the Capture Area will be processed by the CytAssist instrument.

Overlay the slide, centering the tissue on either square.

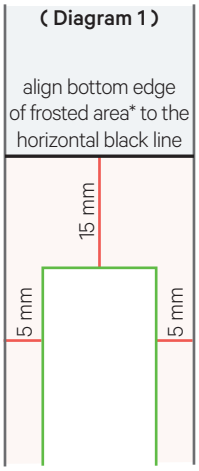
B **If slide has no frosted areas, overlay on this diagram.**

Tissue should lie within the green allowable area:
15 mm from top and bottom edges
5 mm from the sides

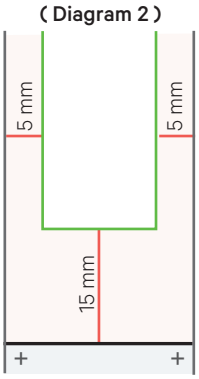


If slide has a frosted end and/or marks, overlay on diagram 1 and then on diagram 2.

Check the allowable area from both the top (1) and bottom (2) to ensure the tissue lies within the green allowable area (*area is variable due to variability in the dimensions of frosted areas across slide brands):*
15 mm from edge of frosted area/marks
5 mm from the sides



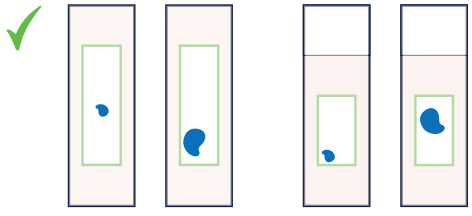
**If text is present below frosted area of slide, align bottom of the text to the line*



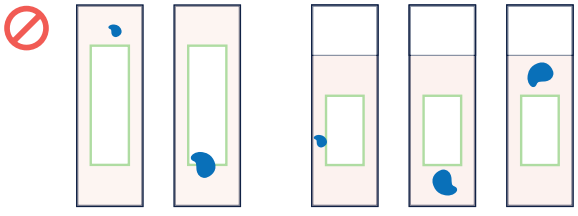
align bottom edge** to the horizontal black line

***If markings are present at bottom edge of slide, align markings to the + signs*

C **Examples of Good Tissue Placement**



Examples of Bad Tissue Placement



Visium CytAssist Tested Slides

The following slides have been tested for use with the Visium CytAssist Tissue Slide Cassette and instrument.

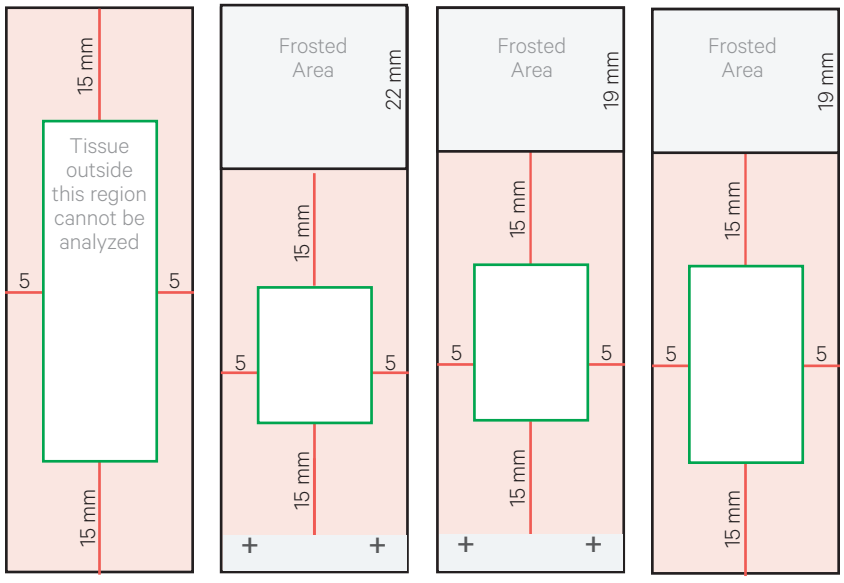
Item	Length (mm)	Width (mm)	Thickness (mm)
Epredia Shandon ColorFrost Plus	75.0	25.0	1.0
Fisherbrand SuperFrost Plus	75.0	25.0	1.0
Sigma-Aldrich Poly Prep Slides	75.0	25.0	1.0
VWR SuperFrost Plus Micro Slide, Premium	75.0	25.0	1.0

If unsure of slide part number, refer to "generic" slide diagram below for general guidance (images not to scale). Diagrams for verifying that tissue sections are placed in the allowable area can also be found in the Visium CytAssist Quick Reference Cards - Accessory Kit (Document CG000548). The diagrams demonstrate allowable areas that are far enough away from frosted sections to not interfere with gasket closure during the CytAssist assay. Frosted sections include the opaque area of the slide as well as any etching on the slide.

While slides are specified as being 25 mm x 75 mm, manufacturing tolerances may lead to dimensions that are too small or large to be compatible with 10x Genomics products. Tissue slide dimensions must be within 24.8 mm - 25.3 mm in width and 74.4 mm - 76.2 mm in length to fit the Visium CytAssist Tissue Slide Cassette.

Minimum slide dimensions: 24.8 x 74.4 mm

Maximum slide dimensions: 25.3 x 76.2 mm



Generic

Epredia Shandon ColorFrost Plus

Fisherbrand SuperFrost Plus

VWR SuperFrost Plus

Sigma-Aldrich Poly Prep

1. Tissue Freezing & Embedding

Overview

This chapter provides guidance on tissue freezing and embedding. Freshly obtained tissue samples must be snap frozen to prevent RNA degradation and avoid ice 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. Other methods of freezing and embedding have not been validated.

Tissue Freezing

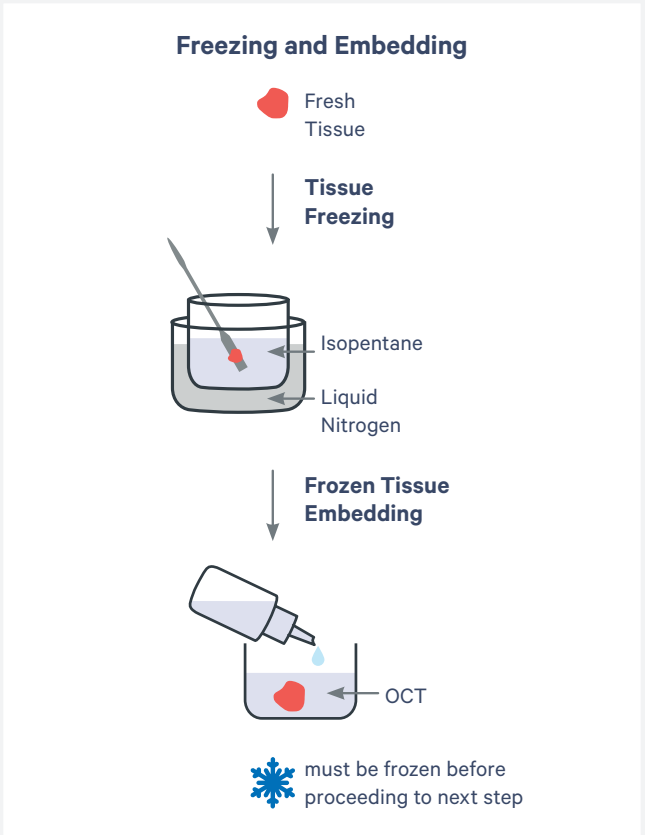
A bath of isopentane and liquid nitrogen is used to freeze freshly obtained tissue. Tissue should not be placed directly in liquid nitrogen as the temperature difference may cause boiling on the surface of the tissues, leading to air pockets and uneven freezing. This may crack and morphologically damage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

Frozen Tissue Embedding

After freezing and prior to cryosectioning, tissue samples are embedded in OCT on dry ice or in isopentane. 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.
- Compatible with multiple staining procedures due to its water solubility.



1.1 Specific Reagents and Consumables

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
Spatula	Round/Tapered Spatula, Stainless Steel	VWR	82027-490
Beaker	Stainless Steel Beaker (250 ml)	VWR	89075-592
Cryovial	WHEATON 5 ml CryoELITE Tissue Vial	Wheaton	W985100
Morter	Liquid Nitrogen Cooled Mini Morter, 1.5 mL Capacity Remove 1.5 mL tube holder prior to use. *Choose appropriate dewar size based on the size of the steel beaker used.*	Grainger	21TT88
Frozen Tissue Embedding			
Item	**Alternatives/Options**	**Vendor**	**Part Number**
OCT	TissueTek O.C.T. Compound	VWR	25608-930
Molds	Disposable Based Molds (15 x 15 mm) *Dependent on the tissue size*	VWR	60872-488
Additional Materials			
Razor blades		-	-
Liquid Nitrogen		-	-
Dry ice		-	-

1.2 Tissue Freezing

Items		Preparation & Handling
Prepare		
☐	Isopentane and liquid nitrogen bath OR dry ice	Fill two-thirds of a metal beaker with isopentane (sufficient to fully submerge the tissue) and place in a liquid nitrogen dewar (same level as isopentane) to allow sufficient contact. Incubate 5-10 min. Isopentane will slowly freeze if left too long in the liquid nitrogen bath. When this occurs, briefly remove the metal beaker containing isopentane from the liquid nitrogen bath. Once thawed, place the beaker back in the liquid nitrogen bath. **DO NOT** use the same isopentane bath for different tissue types.
☐	Tissue	Using a rolled up laboratory wipe, absorb excess blood or solution from the surface of the tissue to limit ice crystal formation. Tissue cracking may occur if tissue has been left in buffer for extended periods.
☐	Pre-cooled Cryovial	Pre-cool a WHEATON CryoELITE cryovial on dry ice.

a. Using either forceps or a spatula, lower the tissue into the isopentane until fully submerged. Keep tissue submerged for ~1 min or until frozen. The freezing time may vary based upon the tissue type and size.

b. Once frozen, transfer the tissue to the pre-cooled WHEATON CryoELITE cryovial on dry ice.

c. 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, the snap-frozen tissue block must be stored in a sealed container.

1.3 Frozen Tissue Embedding

Items		Preparation & Handling
Prepare		
☐	Powdered dry ice	Use a mortar and pestle to prepare powdered dry ice.
☐	Isopentane bath *(alternative to powdered dry ice)*	Fill two-thirds of a polypropylene beaker with isopentane (sufficient to fully submerge the cryomold) and place in dry ice (same level as isopentane) to allow sufficient contact. Incubate for 15 min.
☐	Pre-cooled OCT	Place OCT on wet ice for ≥30 min.
☐	Pre-cooled forceps	Place forceps in dry ice for ≥30 min.
Confirm		
☐	Cryomold	The cryomold 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 and place at room temperature.

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

b. Remove cryovial containing frozen tissue from –80°C (if tissue was stored after step 1.2) and transfer in dry ice.

c. Fill the cryomold with pre-cooled OCT without introducing bubbles.

d. Using pre-cooled forceps, place the frozen tissue into the OCT, covering any exposed surfaces with additional OCT. Confirm there are no bubbles, especially near the tissue.

e. Immediately place the cryomold containing tissue and OCT on powdered dry ice or isopentane bath. If using isopentane, lower the cryomold containing tissue into the isopentane without fully submerging.

f. Wait until the OCT is completely frozen.

STOP

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

A WHEATON CryoELITE cryovial or a resealable bag should be used for storing the tissue block. Remove the tissue block from the cryomold and if needed, trim it using a razor blade to fit into the cryovial.

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

Frozen Tissue Embedding

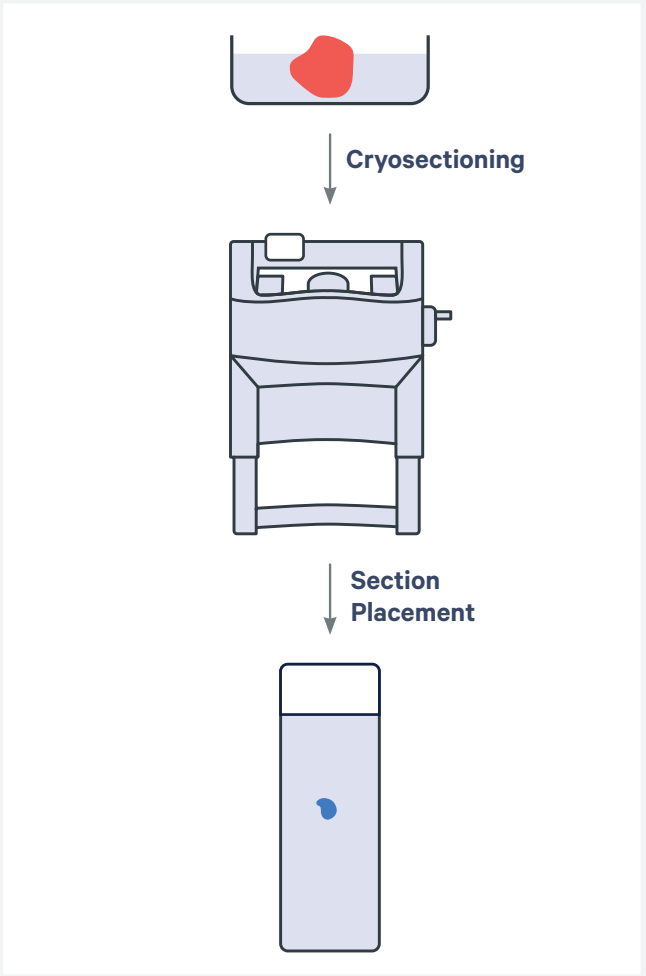
Tissue in OCT

After Embedding

2. Cryosectioning & Section Placement

2.0 Overview

This chapter provides guidance on cryosectioning of the OCT embedded tissue and placement of the tissue sections on blank slides.



Cryosectioning

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

RNA Quality Assessment

It is recommended to assess RNA quality of the tissue block at this stage by calculating RNA Integrity Number (RIN) of freshly collected tissue sections. RNA quality assessment should be done before placing the tissue sections on blank slides. Use sections from the tissue block.

Refer to RNA Quality Assessment for details. RIN should be ≥ 4. Low RIN scores do not necessarily result in poor data, but high scores are more likely to correlate with good data. Various factors could lead to low RIN scores, such as specific tissue types, diseased or necrotic tissues, ischemic tissue, and suboptimal sample preparation and handling.

Section Placement

Tissue sections are placed within the allowable area on compatible blank slides. Placing only one section per slide is recommended.

2.1 Specific Reagents & Consumables

Cryosectioning			
Item	**Alternatives/Options**	**Vendor**	**Part Number**
OCT	TissueTek O.C.T. Compound	VWR	25608-930
Centrifuge Tubes	Sterile Centrifuge Tubes with Flat Caps, 50 ml	VWR	82018-050
Brushes	Magnetic Brush, big	VWR	334172
	Brush, small beveled	VWR	14071425
	Flat cryostat brush, 10 mm	Fisher Scientific	14-071-00
Cryostat	CryoStar NX70 Cryostat *Vacutome, Low Profile Blade Carrier*	Thermo Fisher Scientific	957020
Speciman Chuck	Thermo Scientific CryoStar NX70 Specimen Chuck	Fisher Scientific	14-071-413
Slide Mailer	Simport Scientific LockMailer Tamper Evident Slide Mailer (Alternatively, use a 50-ml centrifuge tube)	Fisher Scientific	22-038-399
Microtome Blade	MX35 Ultra Microtome Blade *Low Profile*	Fisher Scientific	3051835
Glass Anti-Roll Plate, *Optional*	Glass Anti-Roll Plate	Fisher Scientific	A78930200
Blank slides	Epredia Shandon ColorFrost Plus Slides	Fisher Scientific	6776214
	Fisherbrand Superfrost Plus Microscope Slides	Fisher Scientific	12-550-15
	Poly-Prep Slides	Millipore Sigma	P0425
	VWR Superfrost Plus Micro Slides	VWR	48311-703
Additional Materials			
Forceps		-	-
Dry ice		-	-

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.

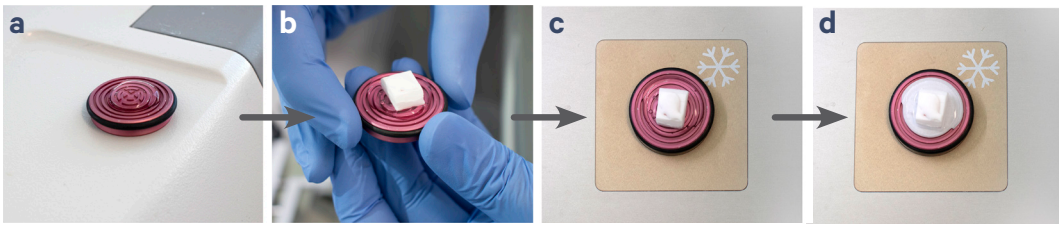
Function	Notes
Main Cryochamber	Maintains stable temperatures from –10°C to –20°C
Cryostat Blade	Separate and adjustable temperature control Maintains stable temperatures from –35°C to –5°C
Specimen Head	Separate and adjustable temperature control Maintains stable temperatures from –50°C to +10°C X-axis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

2.2 Cryosectioning

Items	Preparation & Handling
Adjust	
☐ **Cryostat temperature settings**	Turn cryostat on to pre-cool chamber. Recommended sectioning temperature is –20°C for cryostat blade and –10°C for the specimen head. Follow manufacturer's manual for detailed operations.
Equilibrate	
☐ **Blank slides to the cryostat chamber temperature**	Slides should be cooled down to cryostat temperature for ≥30 min. Warm slides will lead to quick melting of the sections and degradation of RNA.
☐ **OCT embedded tissue block to cryostat chamber temperature**	Freshly prepared or OCT embedded tissue block stored at –80°C must be equilibrated to cryostat chamber temperature for at least 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.

Mount OCT Embedded Tissue Block on the Specimen Stage:

OCT Embedded Tissue Block Mounting



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

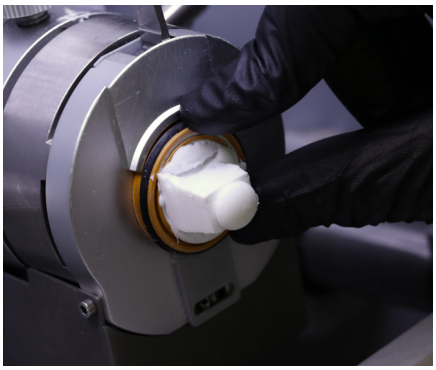
c. Place the stage and the tissue block on the cryobar inside the cryostat chamber.

d. Allow the OCT and the tissue block to freeze and adhere to the specimen stage.

Exposing the Tissue:

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

Install Block onto Specimen Head

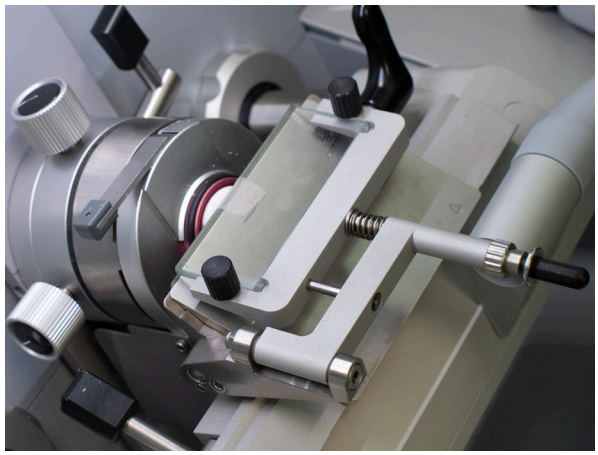
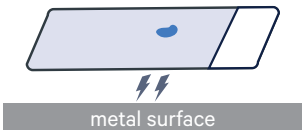


f. Sectioning conditions vary across different tissues and cryostats. Follow manufacturer's recommendation for cryosectioning.

g. Continue sectioning until the tissue is visible.

h. Acquiring sections from the tissue block for RNA quality assessment is recommended. This can be done prior to or after placement of tissue onto blank slides. Refer to step 2.4 RNA Quality Assessment for more information.

2.3 Section Placement

Items	Preparation & Handling
Confirm	
☐ **Section thickness setting**	Recommended section thickness is 10 µm, but tissue section thicknesses of 10–20 µm have been validated with the assay.
☐ **Anti-roll plate is in place,** *(Optional)*	Anti-roll plate prevents rolling of tissue sections. 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.
☐ **Slide electrostatic discharge**	If using a non-charged slide, prior to collecting the tissue section, ensure that the slide has undergone electrostatic discharge with a common metal within the cryochamber to minimize the negatively-charged tissue section from repelling. Allow slides to cool within the cryochamber for 5 min before mounting of the section. 
☐ **Slide storage**	Pre-cool slide mailer or 50-ml centrifuge tube to cryostat temperature for 10-15 min.
Practice	
☐ **Section placement on a blank slide**	Create a representative allowable area on a 75 x 25 x 1 mm blank slide. Optimize section quality and practice section placement within the allowable area before working with experimental blocks. Refer to Determining Allowable Area.

Acquire Section and Place on Blank Slide

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 allowable area on the pre-equilibrated blank slide by gently touching the section with the front 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 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.

Immediately place the slide with tissue section on the cryobar to freeze the section. Continue transferring sections on remaining slides.

d. Transfer the slides containing tissue sections to a pre-cooled slide mailer. Alternatively, use a pre-cooled 50-ml centrifuge tube.

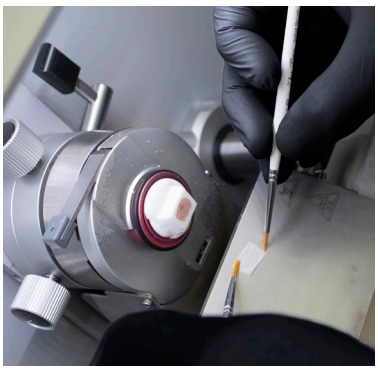
Store slides individually (one slide per 50-ml centrifuge tube, if using) in a sealed container. If using an unsealed slide mailer, store in a secondary sealed container, such as a resealable bag.

e. Transfer slides within slide mailer or centrifuge tube to dry ice.

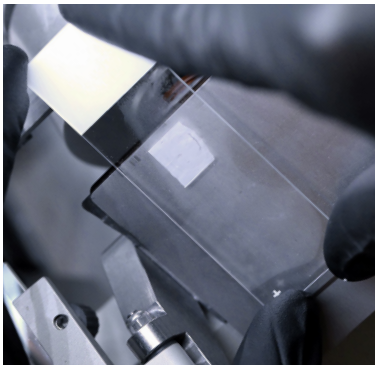
STOP

f. Store slides at –80°C for up to 2 months or immediately proceed to Visium CytAssist Spatial Gene Expression for Fresh Frozen – Fixation, H&E Staining, Imaging & Destaining Demonstrated Protocol (CG000614).

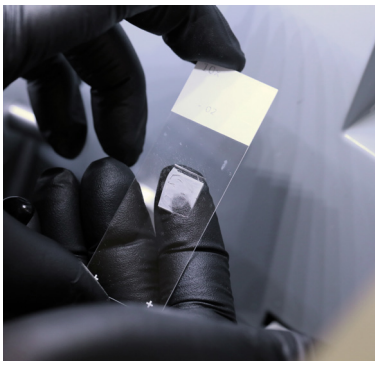
Flatten the Section



Transfer the Section



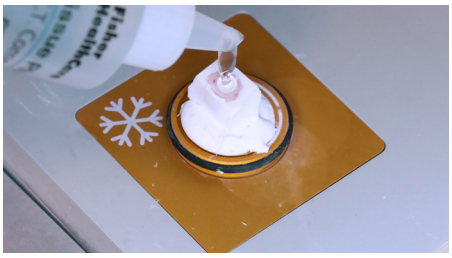
Adhere the Section



Immediately place the slide on the cryobar to allow section to freeze

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 pre-cooled OCT and allow to freeze.



- The frozen tissue block can be stored attached to the specimen stage in a sealed container at –80°C. 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 fully melt, as this will severely damage the tissue. Separation of the tissue block from the specimen stage is optional.
- 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.

Slide Shipping:

- Place slides in a slide mailer and keep cold. If multiple slides are being shipped, ensure that there is sufficient space in between the slides to avoid contact. If placing a slide in the last slot, ensure slide does not face outward.

- Place the mailer in a tightly sealed secondary container to limit exposure and keep cold.
- Samples can be shipped overnight in dry ice, provided there is enough dry ice to account for transit and delivery times.
- Refer to the local institution or delivery service for detailed instructions on shipping samples in dry ice.

2.4 RNA Quality Assessment

This section provides guidance on assessing the quality of the OCT embedded tissue blocks by calculating its RNA Integrity Number (RIN).

RNA Extraction			
Item	**Alternatives/Options**	**Vendor**	**Part Number**
RNA Isolation Kit	RNeasy Mini Kit (50) *Refer to Purification of Total RNA from Animal Tissues in RNeasy Mini handbook*	Qiagen	74104
	QIAshredder (50), *Optional*	Qiagen	79654
	CELLDATA RNAstorm Fresh Cell and Tissue RNA Isolation Kit	Biotium	CD504
Centrifuge Tubes	DNA LoBind Tubes, 1.5 ml	Eppendorf	022431021
2-Mercaptoethanol	2-Mercaptoethanol	Millipore Sigma	M6250-100ML
Ethanol *(if using CELLDATA protocol)*	Ethyl Alcohol	Millipore Sigma	M7023-500ML
Bioanalyzer/ Tapestation Choose Bioanalyzer or TapeStation based on availability & preference.	RNA 6000 Pico Kit	Agilent	5067-1513
	2100 Bioanalyzer Laptop Bundle	Agilent	G2953CA
	4200 TapeStation	Agilent	G2991AA
	High Sensitivity RNA ScreenTape	Agilent	5067-5579
	High Sensitivity RNA ScreenTape Ladder	Agilent	5067-5581
	High Sensitivity RNA ScreenTape Sample Buffer	Agilent	5067-5580
Additional Materials			
Nuclease-free Water		-	-
Razor blades		-	-
Forceps		-	-
Dry ice		-	-
Wet ice		-	-

RNA Quality Assessment

Pre-cool microcentrifuge tube, cooling block, and forceps in cryostat chamber or at –20°C to prevent premature melting of tissue sections.

a. Cryosection 20–30 mg of tissue sections from the OCT embedded tissue block (~4 sections at 25 µm thickness). If tissues contain extensive connective or adipose tissue, cryosection up to 50 mg of tissue.

b. If OCT is excessive (≥1 mm surrounding the tissue), remove excess OCT with a razor blade or with cooled forceps.

c. Using the cooled forceps, transfer sections to a pre-cooled microcentrifuge tube.

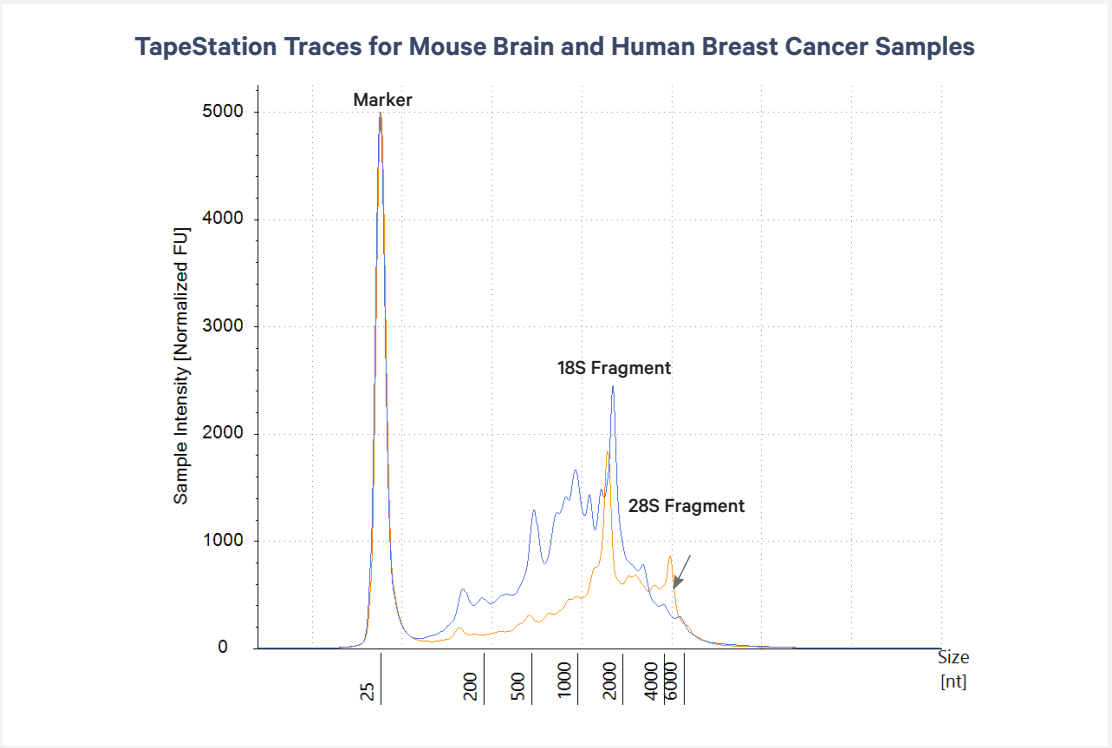
d. Place the pre-cooled microcentrifuge tube containing sections on dry ice. Store at -80°C or proceed to RNA extraction. *DO NOT allow samples to melt, as this will lead to degradation of RNA and a poor RIN score.*

e. Isolate RNA according to manufacturer's instructions. After RNA isolation, place sample on wet ice.

f. Store purified RNA at –80°C for long-term storage or immediately proceed to RIN calculation using either BioAnalyzer or TapeStation reagents. Follow manufacturer's instructions for RIN calculation.

RIN Trace Examples

This section contains example traces for RNA Integrity Number (RIN) assessment.
This protocol was optimized using samples with RIN ≥4.



Samples displayed above are from intact (mouse brain, yellow, RIN = 7;) and degraded (human breast cancer, blue, RIN = 4.4) RNA. The CELLDATA RNAstorm Fresh Cell and Tissue RNA Isolation KIt was used for RNA isolation, while the TapeStation High Sensitivity RNA Screentape kits was used for RIN calculation.

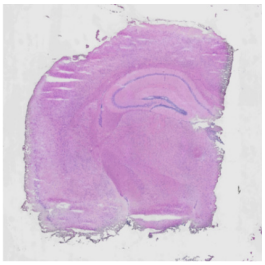
Troubleshooting

Impact of Cryostat Specimen Head Temperatures on Tissue Tearing

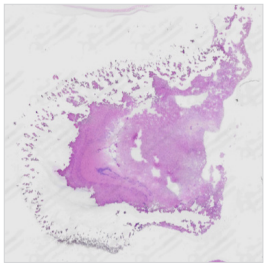
-10°C | -14°C | -20°C | -30°C

Normal Section

Impact of Condensation on Tissue Sections

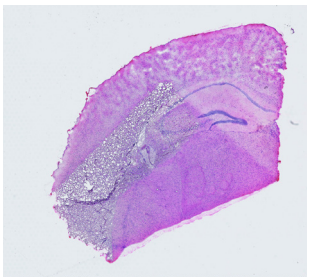


No Condensation. Intact tissue section.

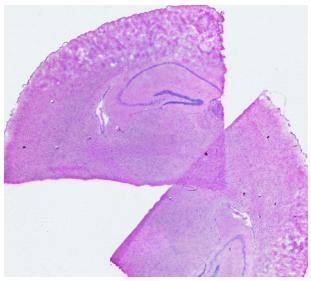


Tissue degraded due to condensation. Always transport slides with tissue sections on dry ice to avoid condensation. DO NOT leave slides at room temperature, especially with fresh sections as the resulting condensation will cause tissue disintegration.

Incorrect Placement of Tissue Sections



Folded tissue section



Overlapping sections

Practice correct section placement on blank slides before proceeding with slides that will be used for the Visium CytAssist Spatial Assay. See Visium CytAssist Tested Slides for templates.

Document Revision Summary

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