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

Introduction

The Visium CytAssist Spatial Gene Expression for Fixed Frozen assay is designed to analyze mRNA in tissue sections derived from fixed frozen tissue samples. 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 slides before and after cryosectioning.
- Tested blank slides compatible with the Visium CytAssist Spatial Gene Expression assay.
- Fixing, cryopreserving, and embedding of tissue samples prior to cryosectioning.
- Cryosectioning of tissue samples and placement of sections on blank slides.
- Assessment of tissue block RNA quality.

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 tissues not tested.

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

- Visium CytAssist Spatial Gene Expression for Fixed Frozen – Rehydration, H&E Staining, Imaging & Decrosslinking Demonstrated Protocol (CG000662)
- Visium CytAssist Spatial Gene Expression User Guide (CG000495), Rev D 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 –10°C to –20°C for blade and –10°C to –20°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. Thicker sections require a 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. Sections outside of that range may result in reduced performance.

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 sections on multiple tissue slides, ensure that sections are placed in the same location on the tissue slides for improved imaging efficiency.
- Only one section from each tissue slide can 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.

RNA Quality Assessment

- Assess RNA quality of the tissue block before placement of sections on blank slides by calculating the percentage of total RNA fragments >200 nucleotides (DV200) of RNA extracted from tissue sections.
- Various factors could lead to variations in DV200 scores, such as specific tissue types, diseased or necrotic tissues, excess OCT, sample preparation, handling, loading concentration errors, or errors with ladder.

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.

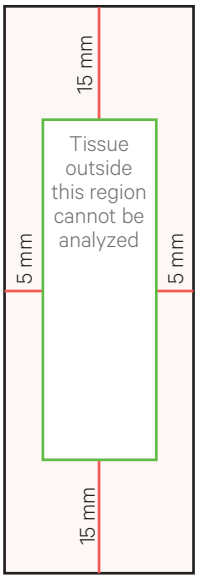
A **Ensure that the area of interest will fit within the Capture Area of a Visium CytAssist Spatial Gene Expression Slide.**

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.

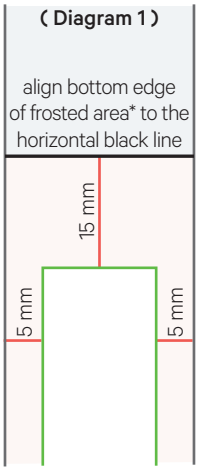
B **If slide has no frosted areas, overlay on this diagram.**

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

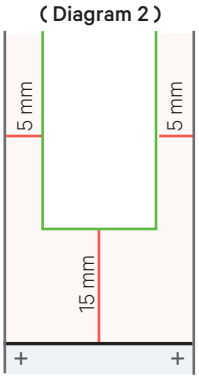


If slide has a frosted end and/or marks, overlay on diagram 1 and then on diagram 2.

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



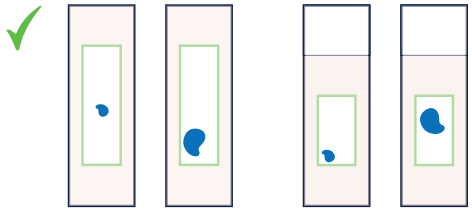
**If text is present below frosted area of slide, align bottom of the text to the line*



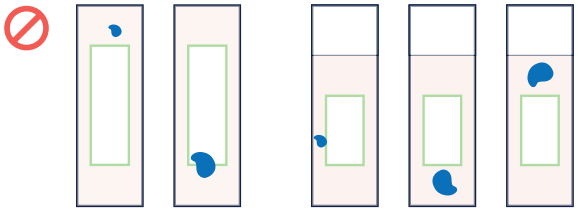
align bottom edge** to the horizontal black line

***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.

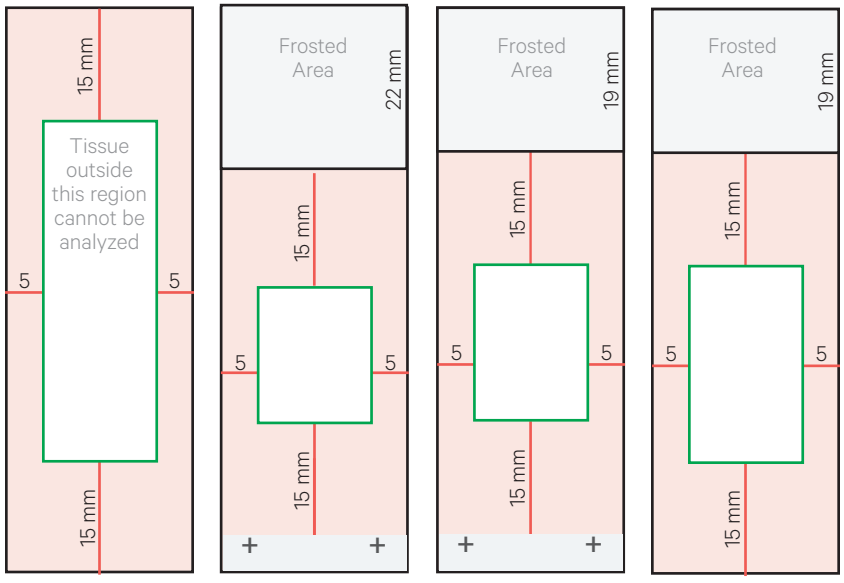
Item	Length (mm)	Width (mm)	Thickness (mm)
Epredia Shandon ColorFrost Plus	75.0	25.0	1.0
Fisherbrand SuperFrost Plus	75.0	25.0	1.0
Sigma-Aldrich Poly Prep Slides	75.0	25.0	1.0
VWR SuperFrost Plus Micro Slide, Premium	75.0	25.0	1.0

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 Fixation & Embedding

Overview

This chapter provides guidance on fixing, cryopreserving, and embedding of tissues. Freshly obtained tissue samples are fixed in a paraformaldehyde (PFA) solution to preserve tissues in their current state. Fixed tissues are then transferred into a cryopreservant sucrose solution to prevent the formation of ice crystals in the tissues. Once fixed and cryopreserved, 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 for fixed frozen samples have not been validated.

Tissue Fixation

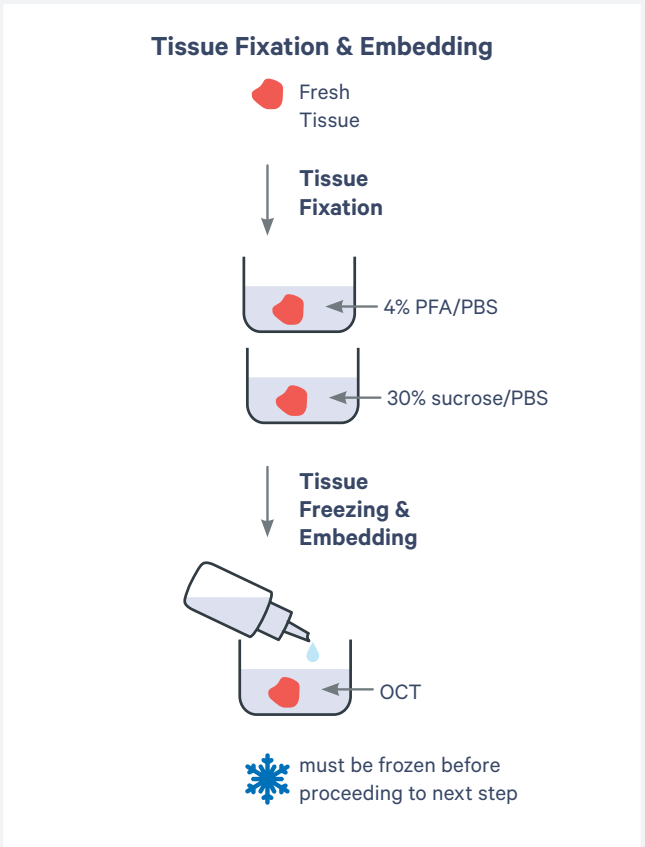
Tissue blocks are fixed in cold 4% PFA solution (pH 7.4) until the tissues sink to the bottom of the solution, approximately 12-16 hours. Tissue block size can impact fixation time. The fixed tissue blocks are then cryopreserved upon placement in 30% sucrose solution until the tissues sink to the bottom of the solution, approximately 6-12 hours. Ensuring that tissues are fully submerged in both fixative and cryopreservant solutions is critical for success.

Tissue Freezing & Embedding

After fixation, and prior to cryosectioning, tissue blocks are simultaneously frozen and embedded in OCT utilizing an isopentane bath or powdered dry ice.

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.



1.1 Specific Reagents and Consumables

Paraformaldehyde should always be used with adequate ventilation in a fume hood. Follow appropriate institutional regulations for handling and disposal of hazardous waste.

Tissue Fixation			
Item	**Alternatives/Options**	**Vendor**	**Part Number**
Paraformaldehyde	Paraformaldehyde	Millipore Sigma	158127
	Paraformaldehyde, 16% w/v aq. solution, methanol free	Alfa Aesar	50-00-0
1X PBS	Phosphate-Buffered Saline, 1X without calcium and magnesium, pH 7.4	Corning	21-040-CV
Sucrose	Sucrose	Millipore Sigma	S0389
HCl	Hydrochloric Acid Solution, 1 N *Or any equivalent HCl*	Honeywell	35328
50-ml Centrifuge Tubes	50 mL centrifuge tubes	Millipore Sigma	CLS430829
Petri Dishes	CytoOne Dishes	USA Scientific	CC7682-3359
Filters	Nalgene Rapid-Flow Sterile Disposable Filter Units with PES Membranes	Thermo Fisher Scientific	565-0020
	Syringe Filter, 0.22µm, sterile	VWR	76479-024
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	Fisherbrand Polypropylene Disposable Beakers	Fisher Scientific	FB0129111
Tissue Freezing & 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
Beaker	Fisherbrand Polypropylene Disposable Beakers	Fisher Scientific	FB0129111
Forceps	Specimen Forceps, Straight, 203 mm (8”)	VWR	82027-436
Cryovial	WHEATON 5 ml CryoELITE Tissue Vial	Wheaton	W985100
Additional Materials			
Dry ice		-	-
pH Meter		-	-
Mortar and Pestle		-	-
Rocker		-	-

1.2 Tissue Fixation

Prepare solutions before harvesting of tissue. Large volumes of 4% PFA and 30% sucrose solutions may be prepared and stored ahead of time. Store 4% PFA at 4°C and 30% sucrose at room temperature.

Items	Preparation & Handling
Prepare	
☐ 1X PBS	Pre-chill large volume of 1X PBS on wet ice or at 4°C prior to use. Following preparation, fill a 50-ml centrifuge tube with 40 ml cold 1X PBS. Prepare one centrifuge tube per tissue. Maintain on ice. Return 1X PBS to wet ice in between use.
☐ 4% PFA/PBS Solution	Weigh 4 g paraformaldehyde in chemical hood. Add 1X PBS to volume of 99 ml. Slowly heat while stirring (DO NOT heat >60°C). Cool to room temperature. Adjust to pH 7.4 with HCl. Add 1X PBS to final volume of 100 ml. If prepared ahead of time, store at 4°C. Alternatively, use commercial 16% paraformaldehyde and dilute to 4% concentration using 1X PBS. Following preparation, fill a 50-ml centrifuge tube with 40 ml 4% PFA solution. Prepare one centrifuge tube per tissue. Maintain on wet ice.
☐ 30% Sucrose/PBS Solution	Weigh 30 g sucrose. Add 1X PBS for final volume of 100 ml. Vortex briefly. Filter using a sterile 0.22 µm filter. If prepared ahead of time, store at room temperature. Following preparation, fill a 50-ml centrifuge tube with 40 ml 30% sucrose solution. Prepare one centrifuge tube per tissue. Maintain at room temperature.

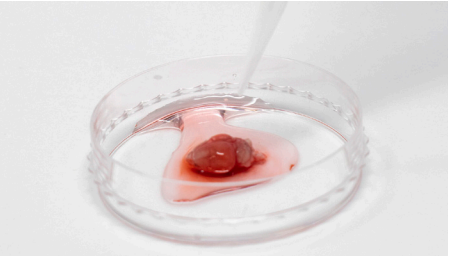
a. Fill bottom of petri dish with cold 1X PBS. Transfer freshly obtained tissue to petri dish using forceps or spatula.

TIPS

Work quickly between tissue harvest and fixation steps.

b. Rinse tissue using a pipette with cold 1X PBS to remove any residual blood.

Tissue Rinse



c. Using either forceps or a spatula, transfer the tissue into a 50-ml centrifuge tube containing freshly prepared, cold 4% PFA/PBS solution.

d. Gently agitate the centrifuge tube containing tissue on a rocker at the lowest setting until tissue sinks to the bottom of the tube, approximately **12-16 hrs** at **4°C.**

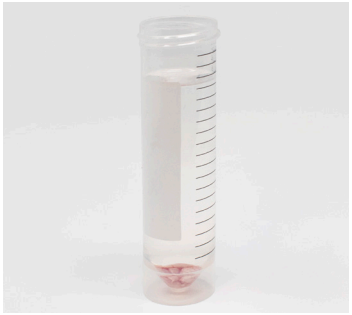
After 12-16 hrs, take tube off rocker and check that tissue has sunk to tube bottom. If not, check every 2-3 hrs for up to 24 hrs.

It is essential that the tissue sinks to the bottom of the solution, indicating proper fixation. Under-fixation can lead to issues with tissue integrity.

Tissue Fixation

Agitation on Rocker

Fully Sunk Tissue



e. Using either forceps or a spatula, transfer the tissue into a 50-ml centrifuge tube containing cold 1X PBS.

f. Incubate for **1 min** at **room temperature**.

g. Carefully discard cold 1X PBS, ensuring the tissue block stays at the tube bottom.

h. **Repeat** cold 1X PBS wash two more times.

Discard 1X PBS

i. Using either forceps or a spatula, transfer the tissue into a 50-ml centrifuge tube containing 30% sucrose/PBS solution and incubate until the tissue sinks to the bottom of the tube, approximately **6-12 h** at **4°C.**

After 6 hrs, check that the tissue has sunk to tube bottom. If not, check every 2-3 hrs for up to 12 hrs.

Tissue Cryopreservation

Transfer Tissue

Fully Sunk Tissue

j. Proceed **immediately** to the next step (Tissue Freezing & Embedding).

1.3 Tissue Freezing & Embedding

Items		Preparation & Handling
Prepare		
☐	Isopentane bath *(preferred method as more rapidly freezes tissue)*	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.
☐	Powdered dry ice *(alternative to isopentane bath)*	Use a mortar and pestle to prepare powdered dry ice.
☐	Pre-cooled OCT	Place OCT on wet ice for ≥30 min.
☐	Pre-cooled forceps	Place forceps in dry ice for ≥30 min.
☐	Pre-cooled cryovial or resealable bag	Place cryovial or resealable bag on wet ice for ≥30 min.
Confirm		
☐	Cryomold	The cryomold used for embedding should be of appropriate size to fit the tissue sample.

Tissue Freezing & Embedding
Isopentane bath setup

a. Label an appropriately sized cryomold to mark the orientation of the tissue and place at room temperature.

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

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

d. Immediately place the cryomold containing tissue and OCT fully submerged in the isopentane bath or on powdered dry ice.

e. Wait until the OCT is completely frozen.

STOP **f.** 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 pre-cooled, 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.*


Tissue Freezing & Embedding
Isopentane bath
Tissue in OCT
Submerge into Bath
After Embedding
Powdered dry ice
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

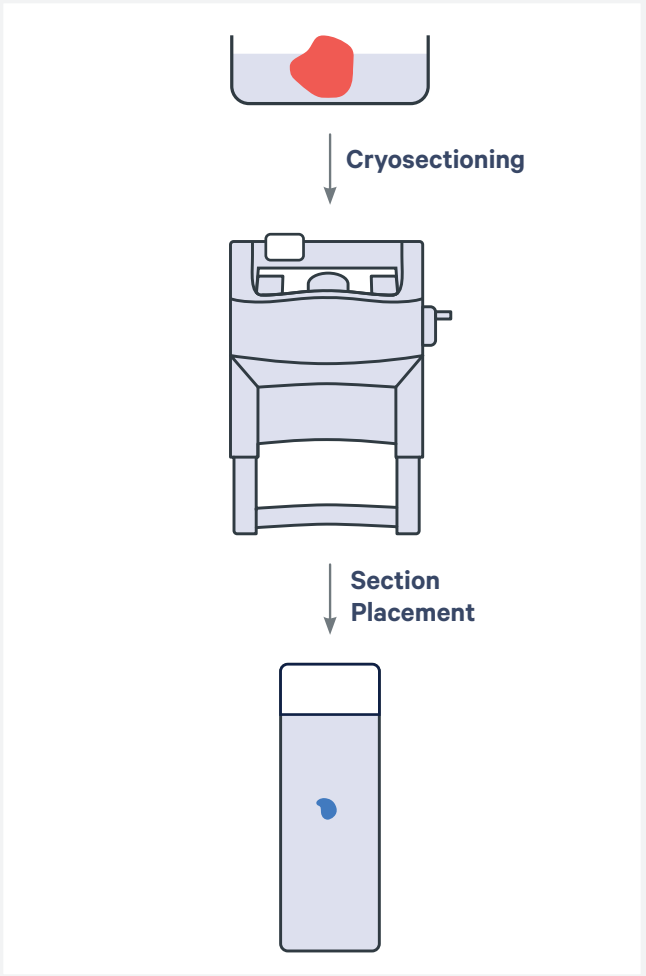
OCT embedded tissue blocks are removed from the –80°C storage and cryosectioned in a cryostat to generate sections for blank slides while keeping samples in a cold environment.

RNA Quality Assessment

RNA quality of the tissue is assessed by calculating DV200 of RNA extracted from OCT embedded fixed frozen tissue blocks. Refer to RNA Quality Assessment for details.

Section Placement

Tissue sections are placed within the allowable area on compatible tissue 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
50-ml Centrifuge Tubes	50 mL centrifuge tubes	Millipore Sigma	CLS430829
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
Specimen 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

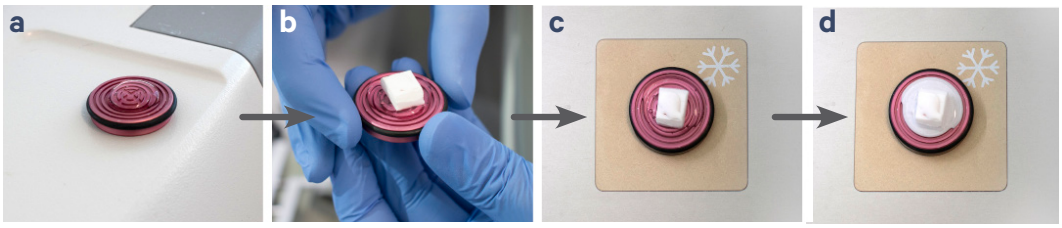
This protocol describes the use of a Cryostar NX70 Cryostat with specific capabilities. Alternatively, use a different cryostat with the following features.

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 setting is –10°C to –20°C for cryostat blade and –10°C to –20°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.

OCT Embedded Tissue Block Mounting



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

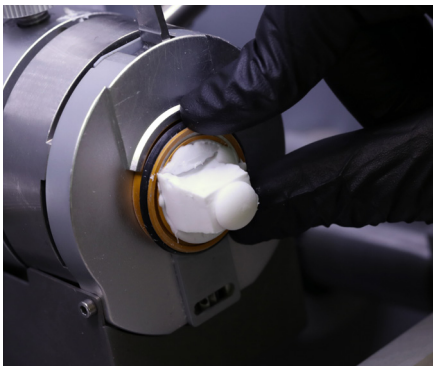
c. Place the stage and the tissue block on the cryobar inside the cryostat chamber.

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

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

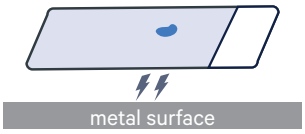
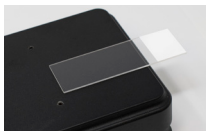


f. Sectioning conditions vary across different tissues and cryostats. Follow manufacturer's recommendation for cryosectioning.

g. Continue sectioning until the tissue is visible.

h. Acquiring sections from the tissue block for RNA quality assessment is recommended. This should be done prior to 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 that has been optimized based upon tissue type. 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**	Validated, charged slides may improve tissue adhesion and are recommended for use in this Demonstrated Protocol. 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 minutes before mounting of the section.  
☐ **Slide storage**	Pre-cool slide mailer or 50-ml centrifuge tube to cryostat temperature for 10-15 minutes before use. If using centrifuge tubes, store only one slide per tube.
Practice	
☐ **Section placement on a blank slide**	Draw 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.

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

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

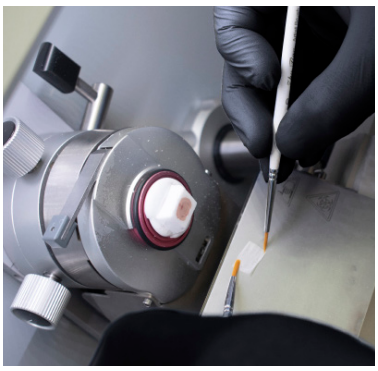
If using 50-ml centrifuge tube, store slides individually. If using an unsealed slide mailer, store in a secondary sealed container, such as a resealable bag.

f. Transfer slides in slide mailer or centrifuge tube on dry ice.

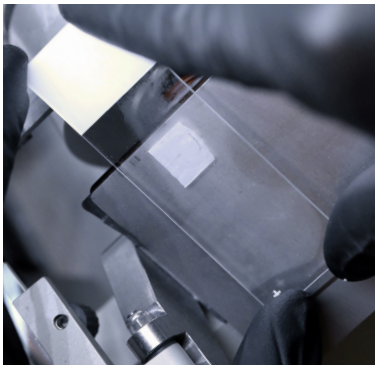
STOP

g. Store slides in sealed slide mailer or centrifuge tube at **–80°C** for up to **2 months** or **immediately** proceed to Visium CytAssist Spatial Gene Expression for Fixed Frozen Tissues – Rehydration, H&E Staining, Imaging & Decrosslinking Demonstrated Protocol (CG000662).

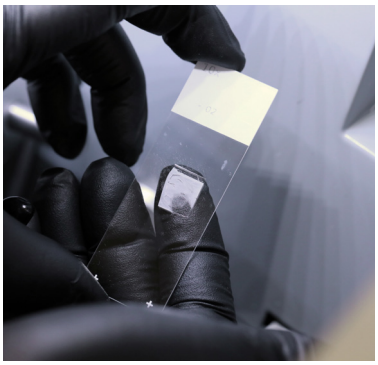
Flatten the Section



Transfer the Section



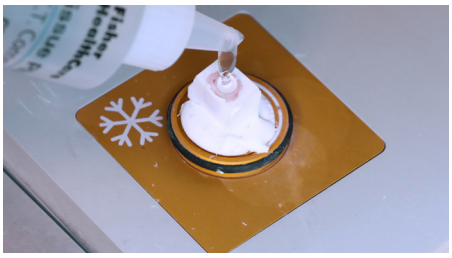
Adhere the Section



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 OCT embedded fixed frozen tissue blocks by calculating its DV200 score. This protocol was optimized using samples with DV200 scores ≥50%.

For RNA Quality Assessment			
Item	**Alternatives/Options**	**Vendor**	**Part Number**
RNA Isolation	CELLDATA RNAstorm FFPE RNA Extraction Kit	Biotium	CD501
RNase Decontamination Solution	RNaseZap RNase Decontamination Solution	Thermo Fisher Scientific	AM9780
Nuclease-free Water	Nuclease-free Water (not DEPC-Treated)	Thermo Fisher Scientific	AM9937
Ethanol	Ethyl Alcohol, 200 Proof	Millipore Sigma	E7023
	Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only)	VWR	83813.360DP
1.5-ml Centrifuge Tubes	DNA LoBind Tubes, 1.5 ml	Eppendorf	022431021
0.2 ml PCR 8-Tube Strips *Choose either Eppendorf, USA Scientific, or Thermo Fisher Scientific PCR 8-tube strips*	PCR Tubes 0.2 ml 8-tube strips	Eppendorf	951010022
	TempAssure PCR 8-tube strip	USA Scientific	1402-4700
	MicroAmp 8-TubeStrip, 0.2 ml	Thermo Fisher Scientific	N8020580
	MicroAmp 8-cap Strip, clear	Thermo Fisher Scientific	N8010535
Nanodrop/Qubit Fluorometer	Nanodrop 2000c Spectrophotometers *Or any equivalent Nanodrop Alternative to Qubit Fluorometer*	Thermo Fisher Scientific	ND-2000C
	Qubit RNA BR Assay Kit	Thermo Fisher Scientific	Q10210
	Qubit Assay Tubes	Thermo Fisher Scientific	Q32856
	Qubit 4 Fluorometer	Thermo Fisher Scientific	Q33238
Bioanalyzer/TapeStation Choose Bioanalyzer or TapeStation based on availability & preference.	2100 Bioanalyzer Laptop Bundle	Agilent	G2953CA
	4200 TapeStation	Agilent	G2991AA
	RNA ScreenTape	Agilent	5067-5576
	RNA ScreenTape Ladder	Agilent	5067-5577
	RNA ScreenTape Sample Buffer	Agilent	5067-5578
Additional Materials			
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 DV200 score.

e. Proceed with RNA isolation starting at **step A5** using the Biotium CELLDATA RNAstorm FFPE RNA Extraction Kit. After RNA isolation, place samples on wet ice.

Fixed frozen tissue is not paraffin embedded and therefore should not undergo the deparaffinization steps as described in steps A1-A4.

f. Store purified RNA at **–80°C** for **long-term** storage or **immediately** proceed to DV200 calculation using either BioAnalyzer, TapeStation, or ScreenTape reagents. Follow manufacturer's instructions (Agilent) for DV200 calculation.

DV200 Performance and Recommendations

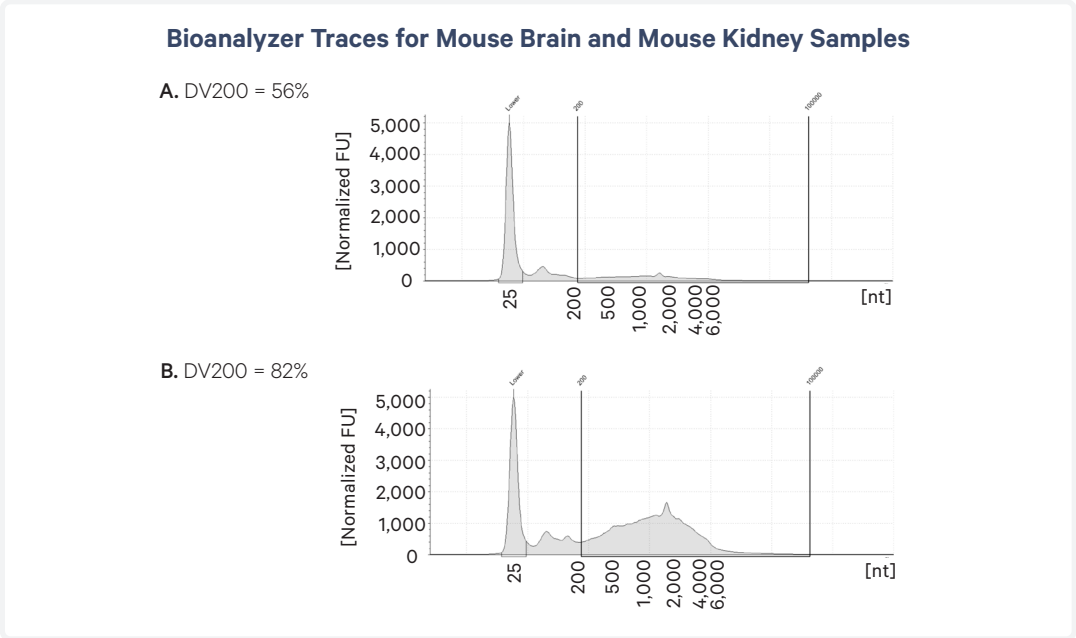
DV200 is a broad measurement of RNA quality and is influenced by factors including:

- Tissue block age, type, and composition
- Region selected for RNA extraction
- Presence of diseased or necrotic regions
- Depth of section
- Fixation method
- Miscellaneous upstream tissue handling and processing

A DV200 score of ≥50% is recommended for the Visium CytAssist Spatial Gene Expression for Fixed Frozen workflow.

Example DV200 Traces

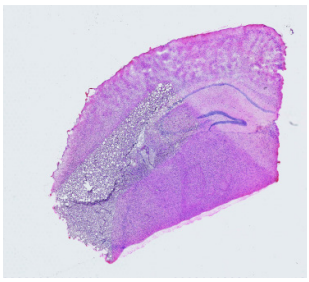
Bioanalyzer traces of RNA extracted from different tissue types for which DV200 was calculated are shown below.



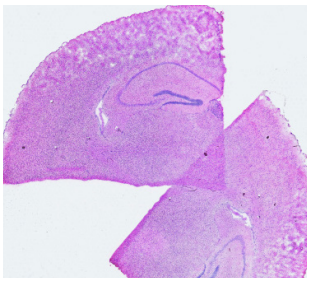
Samples displayed are from Mouse Brain (DV200=56%) (A) and Mouse Kidney (DV200=82)% (B). The Biotium CELLDATA RNAstorm FFPE RNA Extraction Kit was used for RNA isolation, while the TapeStation RNA ScreenTape Kit was used for DV200 calculation.

Troubleshooting

Incorrect Placement of Tissue Sections

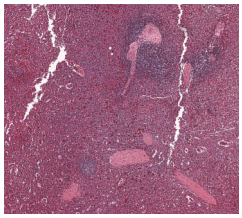


Folded tissue section



Overlapping sections

Cracking of Tissue Sections



Causes

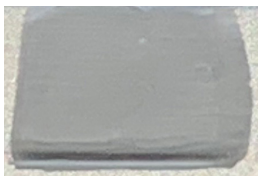
- A tissue block that is too cold can crack while sectioning.
- Sectioning too quickly can lead to cracking.

Troubleshooting

- Reduce sectioning speed and/or increase temperature to reduce cracking.

Impact of Cryostat Specimen Head Temperatures on Tissue Tearing

-10°C

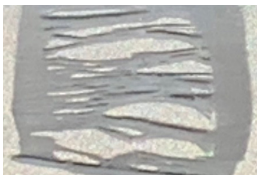


Normal Section

-14°C

-20°C

-30°C



The temperatures shown are representative. Ideal speciman head temperature can vary between tissue types and blocks.

Under-Fixation of Tissue Sections

Ensure tissue block has dropped to tube bottom as recommended. Characteristics of an under-fixed tissue may include:

- Pale or translucent appearance.
- Lack of structure and gelatinous to touch.
- Shrinkage of tissue.

Document Revision Summary

Document Number	CG000663
Title	Visium CytAssist Spatial Gene Expression for Fixed Frozen - Tissue Preparation Guide
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Revision Date	March 2023

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