Visium HD 3' Fresh Frozen Tissue Preparation Handbook

Introduction

The Visium HD 3' Spatial Gene Expression workflow is designed to analyze polyadenylated RNA (poly(A) RNA) in tissue sections derived from fresh frozen (FF) tissue samples. This workflow is facilitated via the CytAssist instrument, which enables the capture of poly(A) RNA onto the Visium HD Slide. A single CytAssist run accommodates up to two stained tissue slides (tissue placed on a blank slide) as sample input. Proper tissue handling and preparation techniques preserve the morphological quality of the tissue sections and the integrity of poly(A) RNA transcripts. Maintaining tissue adhesion and high-quality RNA is critical to assay performance.

This FF Tissue Handbook 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
- RNA quality and optional tissue morphology assessment
- Hematoxylin & eosin (H&E) staining and imaging

Additional Guidance

See the 10x Genomics Support website for additional resources, including a list of tissues tested.

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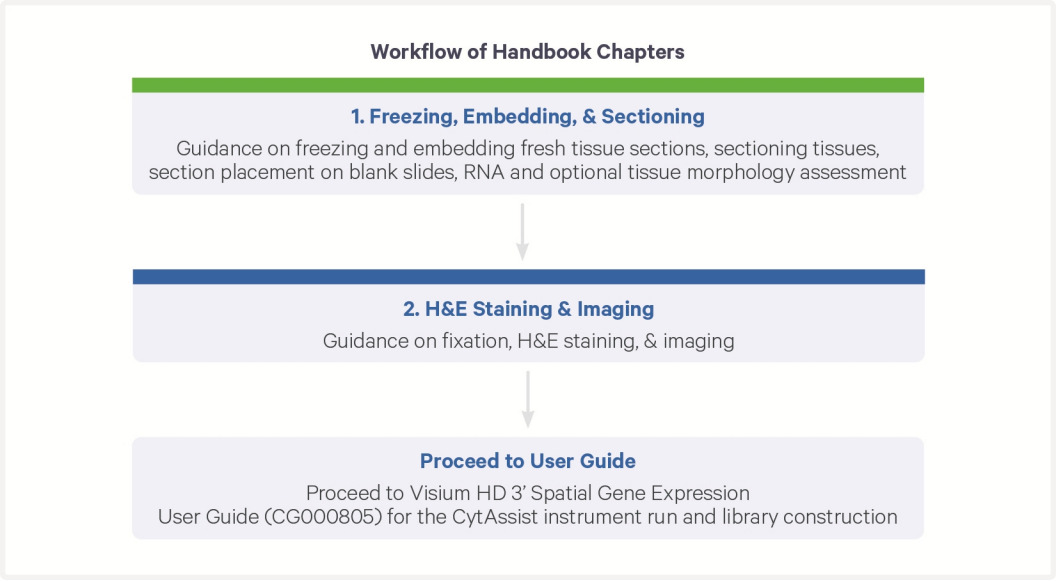
Troubleshooting

Handbook Overview and Navigation

Overview

This handbook describes sample preparation for the Visium HD 3' Spatial Gene Expression workflow. Tabs on the right-hand side of the page denote different sections of this handbook.



Visium HD 3' Spatial Gene Expression Reagent Kits

Consult SDS for handling and disposal information

Visium HD 3' Spatial Gene Expression Reagent Kits

Refer to SDS for handling and disposal information. SDS can be found in the Experimental Design and Planning section ont he 10x Genomics support website.

Visium HD 3' Reagents - Kit A, Small PN-1000854

Visium HD 3' Reagents - Kit A Small
PN-1000854
(store at −20°C)

	#	PN
Enhancer	1	2000482
Reducing Agent B	1	2000087
RNase Inhibitor B	2	2001400
Pre-equilibration Buffer	1	2001399
Perm Enzyme B	1	3000553
Perm Buffer	1	2001398

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*Kit A provides enough reagents for processing four tissue sections through the steps of the Visium HD 3' Spatial Gene Expression User Guide (CG000805). It also includes additional reagents to screen up to four additional tissue sections through H&E staining and imaging.

10x Genomics Accessories

Visium CytAssist Alignment Aid Kit, 6.5 mm PN-1000886

Visium CytAssist Alignment Aid, 6.5 mm
PN-1000886
(store at ambient temperature)

	#	PN
Visium CytAssist Alignment Aid, 6.5 mm	1	3002814

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Visium CytAssist Reagent Accessory Kit PN-1000499

Visium CytAssist Reagent Accessory Kit
PN-1000499
(store at ambient temperature)

	#	PN
10x Magnetic Separator	1	2001212
Low Profile Thermocycler Adapter	2	3000823

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Third-Party Items

Successful execution of the Visium HD 3' workflow requires third-party reagents, kits, and equipment in addition to those provided by 10x Genomics. All third-party reagents and consumables should be obtained prior to starting this workflow.

Consult the Visium HD 3' Spatial Gene Expression Protocol Planner (CG000803) for a list of the following third-party items:

- Additional reagents, kits, and equipment
- Tested pipette tips
- Tested thermal cyclers
- Tested blank slides

10x Genomics has tested all items listed in the Protocol Planner. Unless otherwise noted, 10x Genomics recommends using these items to ensure optimal assay performance. Substituting materials may adversely affect assay performance.

Tips & Best Practices

Icons

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

General Reagent Handling

- Fully thaw and thoroughly mix reagents before use.
- When pipette mixing, unless otherwise specified, set pipette to 75% of total volume. Pipette until reagents are well combined unless a specific number of mixes is specified.

General Best Practices

- When handling tissues, use sterile technique and nuclease-free reagents/consumables.

Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.

RNase-free Environment

- An RNase-free working environment is critical for optimal assay performance.
- An RNase decontamination solution should be used to clean workspaces and equipment.
- Clean workspaces and equipment every workday during the protocol.
- Use new plastic equipment (e.g. centrifuge tubes) and clean glassware as described in the protocol.

RNA Quality Assessment

- Assess RNA quality of the tissue block by calculating the RNA Integrity Number (RIN) of RNA extracted from tissue sections.
- A column-based method of RNA isolation should be used. Alternate methods of RNA isolation may affect score.
- Various factors could lead to variations in RIN scores, such as:
  - Specific tissue types
  - Tissue heterogeneity
  - Diseased or necrotic tissues
  - Sample preparation and handling
  - Excess OCT
  - Loading concentration or ladder errors on the RNA QC platform

Cryosectioning Temperature

- Cryosectioning temperature impacts tissue section integrity. Use a temperature setting of –20°C for the blade and –10°C for the specimen head.
- Temperature settings depend upon 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

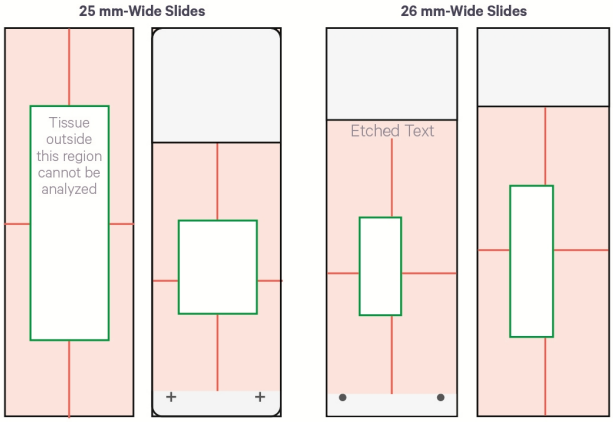
- Sectioning speed depends upon the desired thickness of the sections and the condition of the tissue. Harder and thicker sections require slow sectioning speed.
- Faster sectioning speed may lead to cracks or tears in the sections. Faster section speed may also damage the tissue block or cryostat.

Section Thickness

- Recommended section thickness is 10 µm.
- Sections thinner than the recommended thickness may be difficult to section and result in lower assay performance.
- Sections thicker than the recommended thickness may result in lower data quality.

Section Placement on Blank Slides

- Inspect slides prior to tissue placement for particles and fibers. If found, remove with a lint-free laboratory wipe or compressed air. If using compressed air, do not introduce moisture to the slides.
- After section placement, blank slides are referred to as tissue slides.
- Before section placement, draw an outline of the allowable area on the back of the blank slide to ensure compatibility with the Visium CytAssist instrument, as shown below. Example allowable area images below are not to scale.



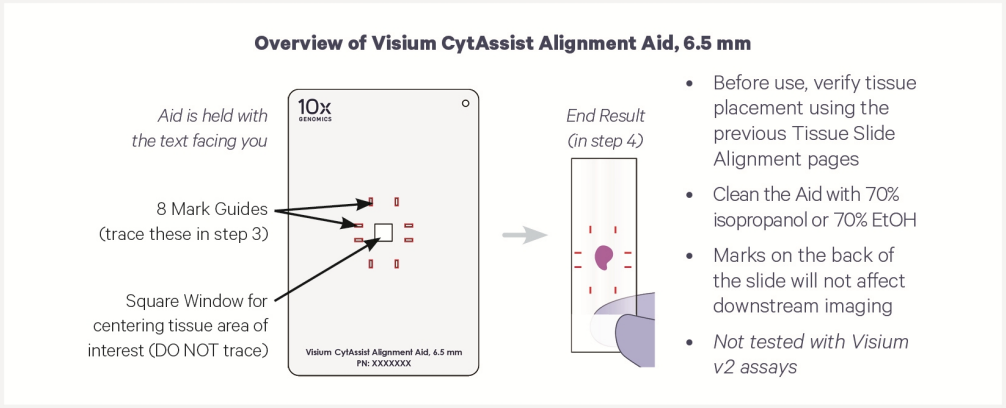
Consult the Visium CytAssist Tissue Slide Alignment Instruction, Quick Reference Cards (CG000548) on the 10x Genomics support website for complete instructions on determining allowable area. Consult the Visium HD 3' Spatial Gene Expression Protocol Planner (CG000803) for a list of tested slides.

- 10x Genomics recommends placing one tissue section per tissue slide.
- Only one section per slide will be captured in the assay.

- Each tissue slide can only be processed with the Visium CytAssist instrument once.

Visium CytAssist Alignment Aid

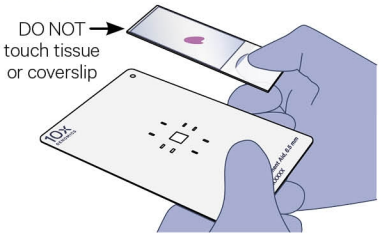
- The Visium CytAssist Alignment Aid (6.5 mm, PN-1000886; available for purchase separately) is an optional accessory used to draw annotations onto the back of the tissue slide to assist with alignment onto the Visium CytAssist instrument.



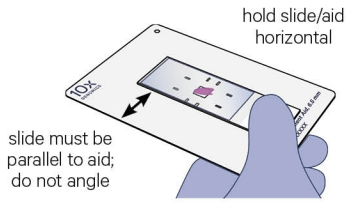
- Use aid after coverslip mounting & imaging and before coverslip removal.
- Ensure back of tissue slide is dry.
- Clean aid with 70% isopropanol or 70% ethanol before use.
- Marks drawn on the back of the slide using the aid will not affect downstream CytAssist imaging.
- Prior to using the aid, remove excess mounting medium by gently touching the slide to a lint-free laboratory wipe.
- Do not move coverslip while using the aid.
- Allow ink to dry for ~5 min.
- Once marks are drawn, avoid wiping the back of the slide vigorously to prevent mark removal. Instead, tap the back of the slide onto a lint-free laboratory wipe to remove excess moisture.

Tissue Slide Alignment on Instrument (using Visium CytAssist Alignment Aid, 6.5 mm)

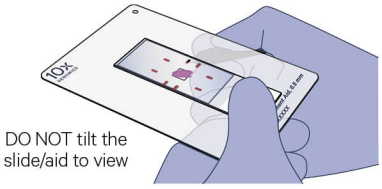
1 Hold the Alignment Aid in one hand horizontally, with the text oriented towards you. In the other hand, hold the tissue slide (tissue facing up) by the frosted end.



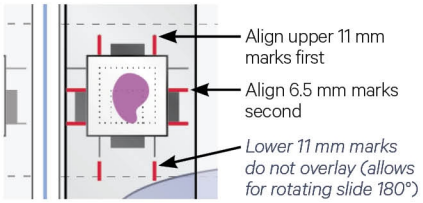
2 Position the slide on top of the aid, with the tissue area of interest centered on the square window. Move thumb to secure the slide against the aid.



3 Select a permanent ultrafine-tipped marker (dark color). Trace through the Mark Guides from the BACK of the Aid onto the back of the slide. DO NOT trace the window. Draw multiple passes for each mark. Allow ink to dry for ~5 min.



4 *Proceed through the protocol steps to Tissue Slide Loading (onto the instrument).* Match the drawn annotations on the back of the tissue slide to the alignment guides on the Tissue Slide Stage.



Practice Section Placement

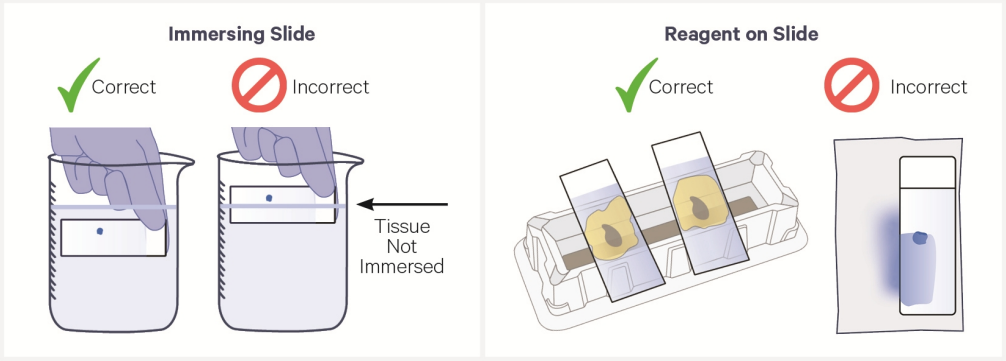
- Practice correct section placement using nonexperimental blocks.
- Sections should be placed on compatible blank slides listed in the Visium HD 3' Spatial Gene Expression Protocol Planner (CG000803).

Handling Tissue Slides

- If a laboratory wipe is required, use 100% polyester lint-free laboratory wipes. Lens paper or non-lint free laboratory wipes are not suitable alternatives. See Visium HD 3' Spatial Gene Expression Protocol Planner (CG000803) for tested part numbers.
- When immersing slides in reagent, ensure all tissue sections are immersed.
- Do not allow tissue slides to dry in between washes, which may impact data quality.
- Maintain tissue slides in a mailer buried in dry ice after removal from the freezer. Do not remove from dry ice until ready to thaw slides on a thermal

cycler during Tissue Slide Preparation.

- When placing a tissue slide 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.
  - Unless tissue slides are being processed together, only place one tissue slide per slide mailer.
  - Immediately place storage container buried in dry ice for transport to a -80°C freezer for long-term storage.
  - Ensure freezer maintains a stable temperature.
  - Avoid tissue slides touching one another while in storage.
- Always wear gloves when handling slides.
- DO NOT touch the tissue sections on the slide.
- Minimize exposure of the slides to sources of particles and fibers.
- Ensure that no absorbent surface is in contact with the reagents on the slide during incubation.



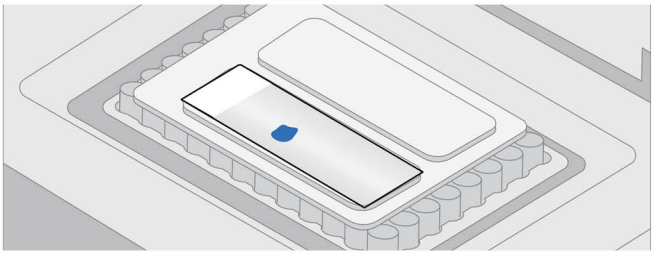
- DO NOT use a hydrophobic barrier (such as one applied by a PAP pen) on slides. These barriers may affect assay performance.
- When imaging, avoid pressing down on the coverslip to ensure easy removal after imaging.

Tissue Slide Incubation

Incubation using a Thermal Cycler:

- Position a Low Profile Thermocycler Adapter on a thermal cycler that is set at the incubation temperature. Low Profile Thermocycler Adapter must come to temperature before placing tissue slide.
- Ensure that the Low Profile Thermocycler Adapter is in contact with the thermal cycler surface uniformly. Consult the Visium HD 3' Spatial Gene Expression Protocol Planner (CG000803) for information on thermal cycler compatibility.
- Position tissue slides on the Low Profile Thermocycler Adapter with the side with tissue facing up.
- Ensure that the entire bottom surfaces of the tissue slides are in contact with the Low Profile Thermocycler Adapter.
- DO NOT close the thermal cycler lid when incubating tissue slides.
- Allow Low Profile Thermocycler Adapter to cool before removing from thermal cycler.

Incubation on a Thermal Cycler



1. Tissue Handling, Freezing, Embedding, Sectioning, Section Placement, and Quality Assessment

1.0 Overview

This chapter provides guidance on tissue freezing, embedding, sectioning, section placement, and quality assessment. Proper tissue handling, storage, and preparation techniques preserve the morphological quality of tissue sections and the integrity of poly(A) RNA transcripts.

Freshly obtained tissue samples must be snap frozen or directly embedded in a freezing and embedding compound, Optimal Cutting Temperature (OCT), to prevent RNA degradation and avoid ice crystal formation. Ice crystal formation may lead to morphological damage of the tissue. OCT helps preserve the structure of the tissue and provides structural support during cryosectioning. Other methods of freezing and embedding have not been validated.

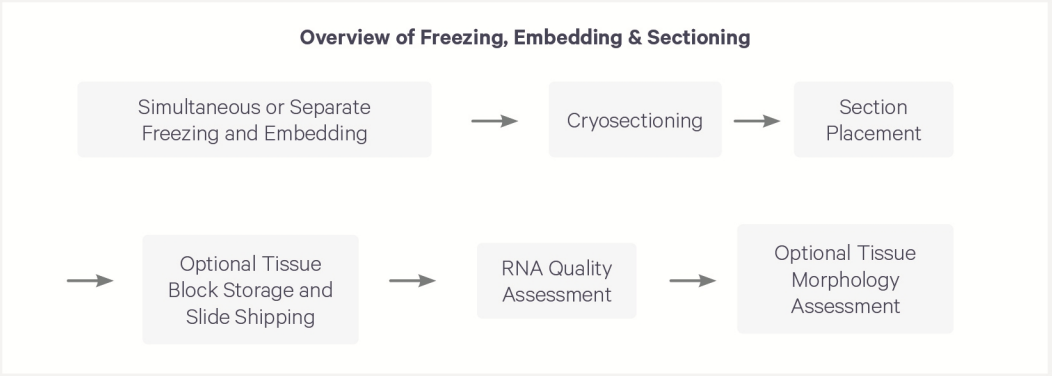
When working with large tissues, segment the tissue at the time of tissue harvest into a smaller size that will fit into the OCT mold. Cut the tissue with a scalpel in a Petri dish containing cold 1X PBS.

To assess RNA quality, RNA Integrity Value (RIN) scores may be calculated from the chosen fresh frozen sample. This measure of RNA quality relies on the assessment of the TapeStation (or similar) electrophoretic trace. Tissue sections with RIN ≥ 7 are optimal for the Visium HD 3' assay. Low RIN scores do not necessarily result in poor data, but high scores are more likely to correlate with higher data quality.

While RIN values provide insights into RNA integrity, it is also important to consider the following key factors that help maintain RNA quality and tissue integrity:

- Gentle handling of fresh tissue
- Minimizing post-mortem intervals
- Washing with RNase-free, cold isotonic storage conditions (such as cold 1X PBS) and removing excess liquid prior to freezing
- Immediate freezing of fresh samples, either via separate or simultaneous tissue freezing and embedding

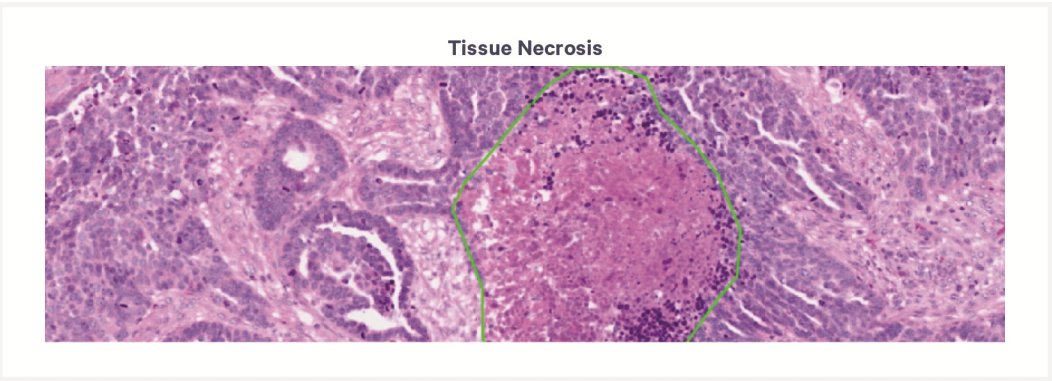
- Assessment of H&E image for signs of necrosis or other impacts to tissue integrity



Tissue Handling

Prior to fixation and embedding, tissues should be handled according to the following guidelines to maximize RNA quality and prevent degradation.

Gentle Handling - Tissues should be handled gently to avoid mechanical stress. Mechanical stress may damage tissue structure. Examples of processes that introduce mechanical stress include ischemia, coagulative necrosis from electrocautery, and hemorrhages from surgical trauma. In the example image below, necrotic regions marked by green lines have signs of nuclear condensation (pyknosis) and nuclear fragmentation (karyorrhexis). These regions are typically characterized by poor RNA quality. The surrounding regions have normal-appearing nuclei.



Minimizing Ischemia/Post Mortem Interval (PMI) - Prolonged ischemia and PMI can negatively affect tissue quality. If processing delays occur, keep tissues in a cold isotonic solution such as cold 1X PBS and avoid exceeding four hours between tissue resection and freezing. However, this allowable time may vary across tissues. For instance, some tissues have higher levels of RNases (lung, pancreas, etc.) and can be more prone to degradation. Long-term storage of fresh tissue in isotonic solutions is not recommended and tissue samples

should be frozen immediately after resection. Delayed tissue freezing may lead to autolysis, degrading tissue, and negatively impacting results.

Frozen Tissue Block Preparation

Freshly obtained tissue can be frozen using two approaches. Both methods aim to preserve tissue morphology and minimize risk of artifacts.

Separate Freezing & Embedding

Tissue is frozen in an isopentane bath pre-chilled with dry ice, then embedded in pre-chilled OCT. This method may also be followed when tissues are already frozen by an external vendor (thus, only embedding in OCT is required).

Simultaneous Freezing & Embedding

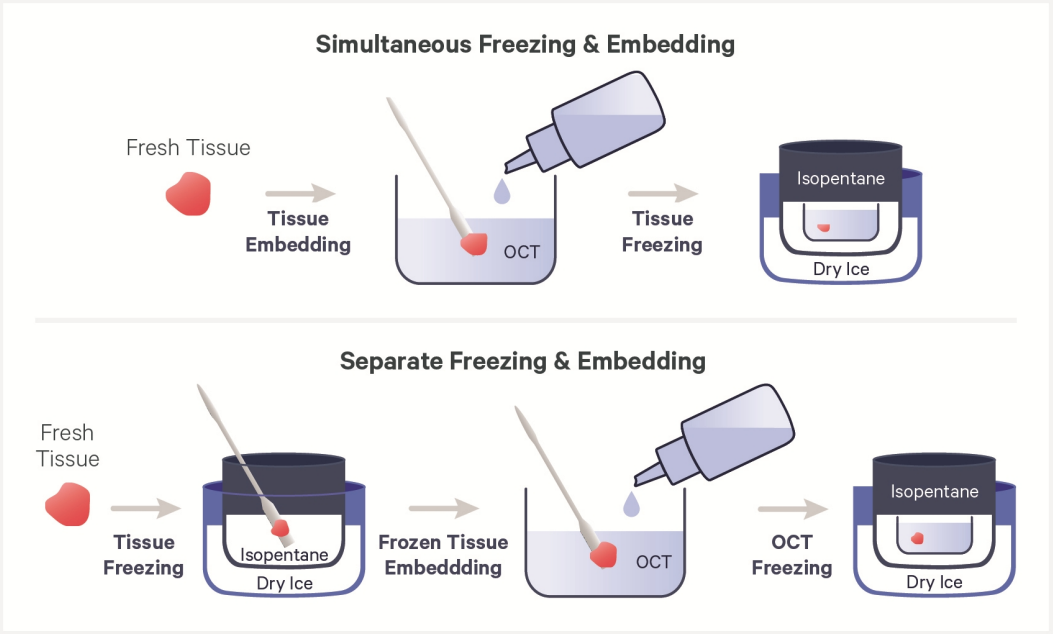
Fresh tissue is embedded directly in pre-cooled OCT and frozen in an isopentane bath pre-chilled with dry ice. This method is suitable when fresh tissue is available and ensures minimal handling. Ensure that the tissue is moisture-free to avoid ice crystal formation that can damage tissue morphology.

If dry ice is unavailable when setting up the isopentane bath, liquid nitrogen can be used as an alternative. However, isopentane pre-chilled with dry ice is preferred over isopentane cooled with liquid nitrogen, as it provides more consistent and uniform freezing temperatures. Using isopentane with liquid nitrogen presents a risk of extremely cold temperatures that may potentially damage tissue morphology.

Never place tissue directly into liquid nitrogen to prevent surface boiling, air pockets, and freezing artifacts.

Prior to cryosectioning, ensure tissues 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.
- Compatible with multiple staining procedures due to its water solubility.



Cryosectioning

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

Section Placement

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

RNA Quality Assessment

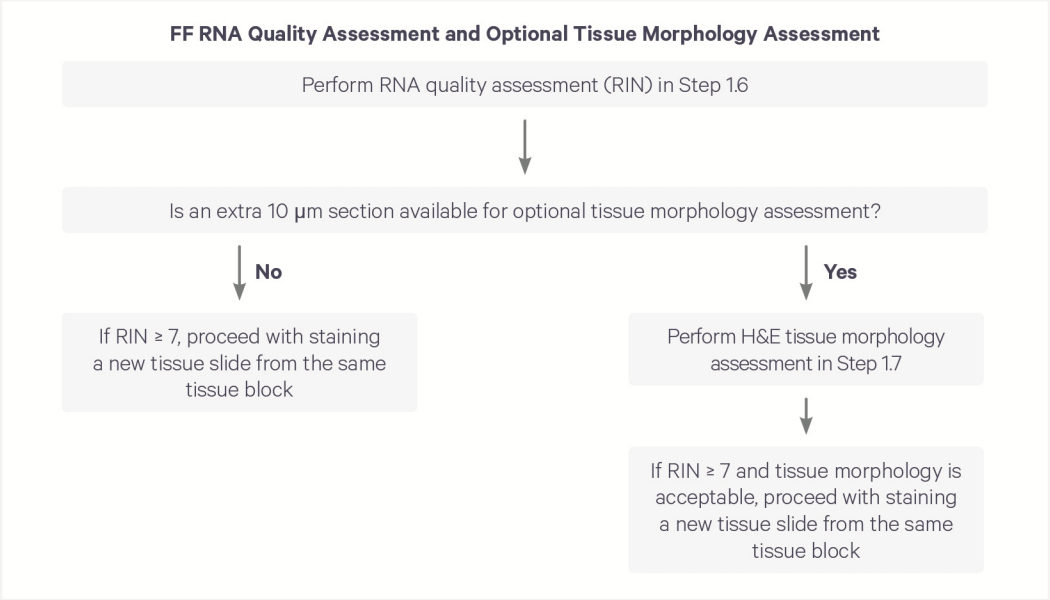
Assess RNA quality of the tissue block at this stage by calculating RNA Integrity Number (RIN) of freshly collected tissue sections. RNA quality assessment should be done before placing the tissue sections on blank slides. Use sections from the same tissue block that will produce sections for the main assay.

See RNA Quality Assessment for details.Tissue sections with RIN ≥ 7 are optimal for the Visium HD 3' assay and typically result in higher data quality. Tissues with lower RIN scores likely will result in lower mapping rates. Tissues with low RIN scores and/or rich in RNases (lung, intestine, pancreas, etc) may have varying impacts to mapping rates and sensitivity metrics depending on the extent of poly(A) RNA degradation.

Low RIN scores do not necessarily result in poor data, but high scores are not an absolute determinant for assay success. Various factors could lead to low RIN scores, such as specific tissue types (diseased or necrotic tissues, ischemic

tissue), suboptimal sample preparation and handling, suboptimal RNA extraction, or errors with the RNA QC platform.

To acquire tissue sections for RNA quality assessment, cryosection **20-30 mg** of tissue sections from 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. If extra sections are available, placing them on slides for Optional Tissue Morphology Assessment is recommended. Use the decision tree below to determine what quality assessment protocols should be followed.



Optional Tissue Morphology Assessment

Assessment of tissue morphology via H&E staining prior to performing the Visium HD 3' assay is recommended. After staining, tissues are assessed to determine suitability for the Visium HD 3' assay.

H&E may be performed on the same tissue section or on serial sections. If extra tissue sections are not available to perform this assessment, H&E images generated later in this handbook may be evaluated to gain additional insights on sample quality.

Estimation of RIN scores as well as the morphological assessments based on H&E help identify potential tissue morphology and RNA quality issues. However, these methods are not all-encompassing and may not always correlate exactly to final assay performance.

1.1 Get Started

Consult the Visium HD 3' Spatial Applications Protocol Planner (CG000803) for a list of third-party items.

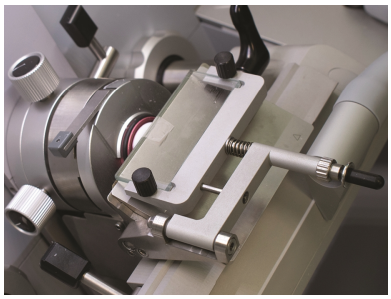
For Freezing & Embedding

Items		Preparation & Handling
Prepare		
☐	Isopentane in dry ice (preferred) OR liquid nitrogen	Fill two-thirds of a polypropylene beaker with isopentane sufficient to fully submerge the tissue (separate freezing & embedding) or cryomold (simultaneous freezing & embedding). Place in dry ice (same level as isopentane) to allow sufficient contact. Incubate 15 min. **Isopentane Bath Setup**  If dry ice is unavailable and the isopentane bath must be prepared with liquid nitrogen, fill two-thirds of a metal beaker with isopentane sufficient to fully submerge the tissue (separate freezing & embedding) or cryomold (simultaneous freezing & embedding). Place in a liquid nitrogen dewar (same level as isopentane). Incubate 15 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	If tissue is fresh, 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 or resealable bag	Pre-cool a WHEATON CryoELITE cryovial or resealable bag on dry ice.
☐	Pre-cooled OCT	Place OCT on ice for ≥30 min.
☐	Pre-cooled forceps	Place forceps on dry ice for ≥30 min.
Confirm		
☐	Cryomold	The cryomold used for embedding should be of appropriate size to fit the tissue sample.

For 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 OCT-embedded tissue block 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.

For Section Placement

Items		Preparation & Handling
Confirm		
☐	Section thickness setting	Recommended section thickness is 10 µm.
☐	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.

Items		Preparation & Handling
Confirm		
☐	Slide storage	Pre-cool slide mailer or 50-ml centrifuge tube (one per tissue slide) to cryostat temperature for 10–15 min.

Items		Preparation & Handling
Practice		
☐	Section placement on a blank slide.	Create a representative allowable area on a blank slide. Optimize section quality and practice section placement within the allowable area before working with experimental blocks.

For RNA Quality Assessment

Items		Preparation & Handling
Equilibrate		
☐	Microcentrifuge tube to cryostat chamber temperature	Microcentrifuge tube should be cooled down to cryostat temperature by placing the microcentrifuge tube in the cryostat chamber for ≥30 min or at -20°C for ≥30 min.
☐	Forceps to the cryostat chamber temperature	Forceps should be cooled down to cryostat temperature by placing the forceps in the cryostat chamber for ≥30 min or at -20°C for ≥30 min.

1.2 Separate Tissue Freezing & Embedding

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

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

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

STOP

c. Transport cryovial or resealable bag on dry ice and store frozen tissue at **-80°C** for **long-term** storage or immediately proceed to the next step.

d. 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. OCT will quickly turn white once frozen, making it hard to determine tissue orientation later.

e. If frozen tissue was stored, remove cryovial or resealable bag containing frozen tissue from **-80°C** and transfer in dry ice.

f. Fill cryomold with pre-cooled OCT without introducing bubbles.

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

h. Immediately lower cryomold containing tissue into isopentane.

i. Wait until OCT is completely frozen.

Freezing OCT

OCT is not frozen (clear)

OCT is frozen (opaque)

STOP

j. Store OCT embedded tissue block in a sealed container and transport on dry ice for storage at **–80°C** for **long-term**, or immediately proceed to 1.4 Cryosectioning on page 25.

A WHEATON CryoELITE cryovial or resealable bag should be used for storing the tissue block. Failure to use a sealed container for storage may

dehydrate and damage the tissue.

If using a cryovial, apply a thin layer of OCT to the tissue to form a protective layer. Allow OCT to freeze. Score the OCT to mark tissue orientation, as this will be lost when tissue block is removed from cryomold. Remove tissue block from cryomold. If needed, trim using a razor blade to fit into the cryovial.

1.3 Simultaneous Tissue Freezing & Embedding

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. OCT will quickly turn white once frozen, making it hard to determine tissue orientation later.

b. Fill cryomold with pre-cooled OCT without introducing bubbles.

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

d. Immediately lower cryomold containing tissue into isopentane.

DO NOT fully submerge cryomold.

e. Wait until OCT is completely frozen.

Freezing OCT

OCT is not frozen (clear)

OCT is frozen (opaque)

STOP

f. Store OCT embedded tissue block in a sealed container and transport on dry ice for storage at **–80°C** for **long-term**, or immediately proceed to 1.4 Cryosectioning on the next page.

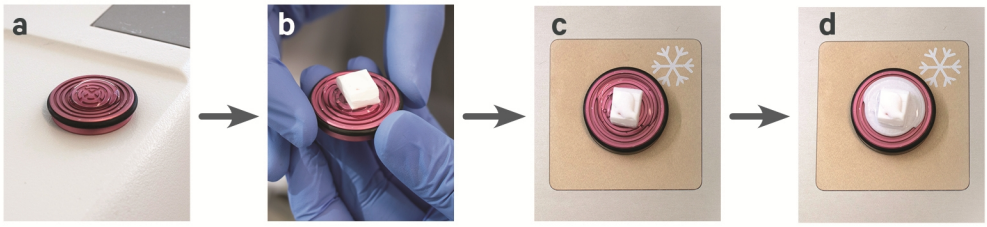
A WHEATON CryoELITE cryovial or resealable bag should be used for storing the tissue block. Failure to use a sealed container for storage may dehydrate and damage the tissue.

When storing a tissue block, apply a thin layer of OCT to the tissue to form a protective layer. Allow OCT to freeze. Score the OCT to mark tissue orientation, as this will be lost when tissue block is removed from cryomold.

Remove tissue block from cryomold. If needed, trim using a razor blade to fit into the container.

1.4 Cryosectioning

OCT Embedded Tissue Block Mounting



a. Fill specimen stage (chuck) with OCT.

b. Ensure OCT-embedded tissue block has been equilibrated to cryostat temperature as described in 1.1 Get Started on page 20

c. Place OCT-embedded tissue block on stage with cutting surface facing away from stage.

d. Place stage and tissue block on cryobar or in cryostat chamber.

e. Allow OCT and tissue block to freeze and adhere to specimen stage.

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

Install Block onto Specimen Head

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

g. Continue sectioning until tissue is visible. Once tissue is visible, subsequent sections may be used for section placement. See Section Thickness on page 10 for information on recommended thickness.

h. Prior to section placement, acquiring sections from the tissue block for RNA quality assessment is recommended. See 1.7 RNA Quality Assessment on page 29 for more information.

1.5 Section Placement

10x Genomics recommends placing one tissue section per tissue slide. However, if placing more than one tissue section on a slide for tissue screening:

- Ensure sufficient spacing between sections to prevent repeated thawing of prior sections
- Ensure that all sections are within the allowable area.

Only one section per slide will be captured in the assay.

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

b. Gently touch section to front of pre-chilled blank slide within allowable area.

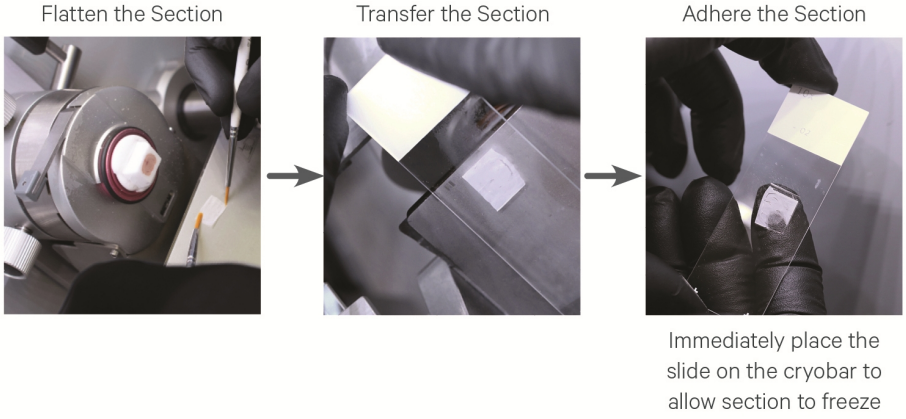
DO NOT place sections on a room temperature slide. Slide should be equilibrated to cryostat chamber.

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

Ensure that entire tissue section is fully adhered.

DO NOT remove slide from cryostat chamber at any point during sectioning and tissue placement.

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



e. If marks were drawn on the slide to mark the allowable area, remove them with a lint-free laboratory wipe before removing from the cryostat.

f. Transfer 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.

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

STOP

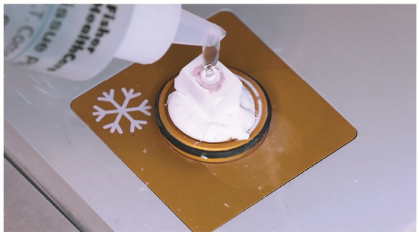
h. Store slides at **–80°C** for up to **4 weeks** or immediately proceed to either optional morphology assessment or tissue staining.

1.6 Tissue Block Storage and Tissue Slide Shipping

Leftover Tissue Block Storage

- Remove leftover tissue block attached to the specimen stage from the cryostat's specimen head and place onto cryobar.
- Cover exposed tissue with a thin layer of pre-cooled OCT and allow to freeze.
- Frozen tissue block can be stored attached to specimen stage in a sealed container at **–80°C**. To separate frozen tissue block from specimen stage, lift tissue block and stage from cryobar and lightly warm stage with hands or an aluminum block at room temperature.
- DO NOT let block and tissue fully melt, as this will severely damage the tissue. Separation of the tissue block from the specimen stage is optional.

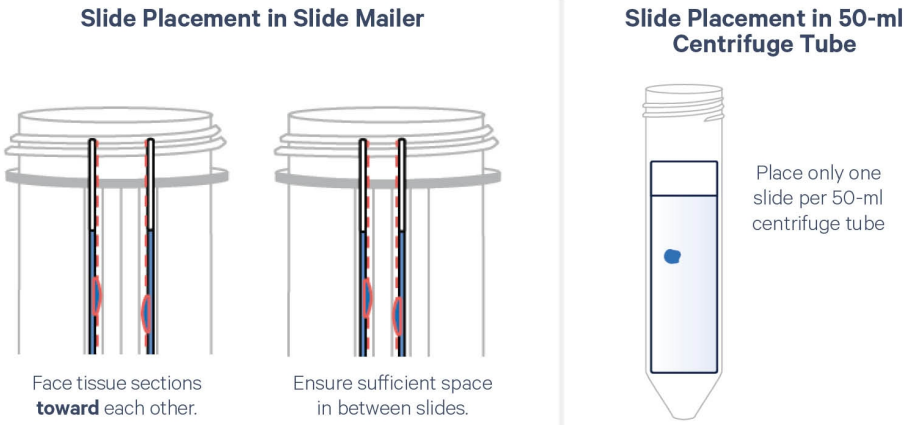
- Immediately place tissue block in dry ice. Ensure that melted areas have refrozen.
- Store in a sealed container at **–80°C** for **long-term** storage.



Slide Shipping

Shipping slides may result in RNA degradation.

- Place tissue slides in a slide mailer or one slide per 50-ml centrifuge tube and keep cold.
- Place mailer in a tightly sealed secondary container.
- Samples can be shipped overnight in dry ice, provided there is enough dry ice to account for transit and delivery times. Ensure that samples are in an environment with a stable temperature.
- See local institution or delivery service for detailed instructions on shipping samples in dry ice.



1.7 RNA Quality Assessment

RNA quality assessment is recommended. If RNA quality will not be assessed, proceed directly to optional tissue morphology assessment or appropriate staining section within this workbook.

a. Cryosection **20-30 mg** of tissue sections from 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.

Excess OCT may reduce RNA quality score.

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

d. Place 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.

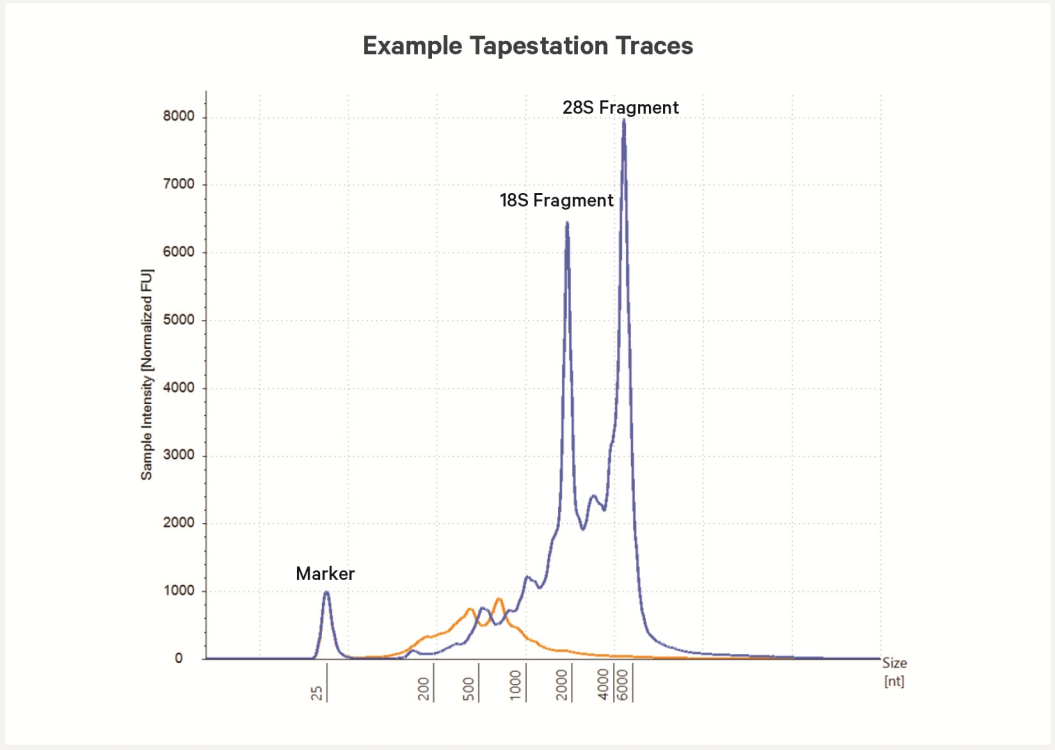
e. Proceed with RNA extraction following manufacturer's instructions (See Visium HD 3' Protocol Planner (CG000803), for tested part numbers). After RNA isolation, place sample on ice.

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.

Ensure RNA is quantified using a Qubit or Nanodrop and loaded within specifications of the relevant assay kit before running RNA on TapeStation or Bioanalyzer.

RIN Trace Examples

This section contains example traces for RNA Integrity Number (RIN) assessment. This protocol was optimized using samples with RIN ≥7.



Samples displayed above are from intact (blue, RIN = 7.5;) and degraded (orange, RIN = 1.5) RNA. The Qiagen RNeasy Mini Kit was used for RNA isolation. The TapeStation High Sensitivity RNA Screentape kits were used for RIN calculation.

1.8 Optional H&E Staining for Tissue Morphology Assessment

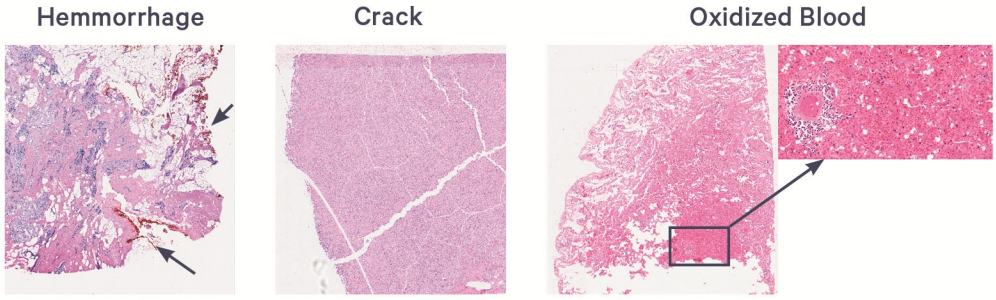
Prior to H&E staining, tissue slide preparation and fixation is required. Prepare slide as described in 2.4 Tissue Slide Preparation on page 42 and fix as described in 2.5 Fixation on page 43.

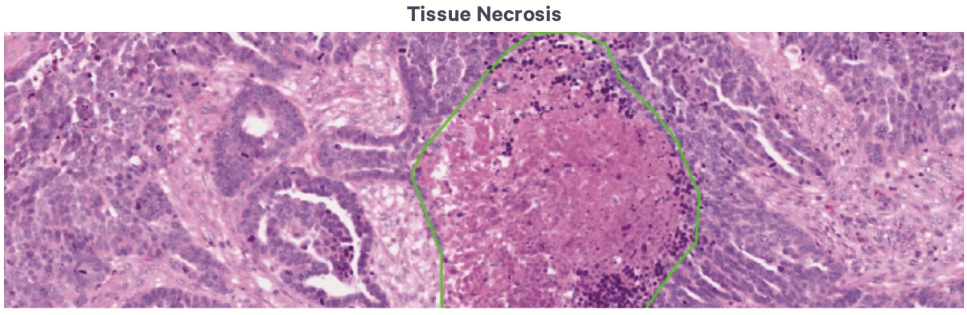
H&E Staining

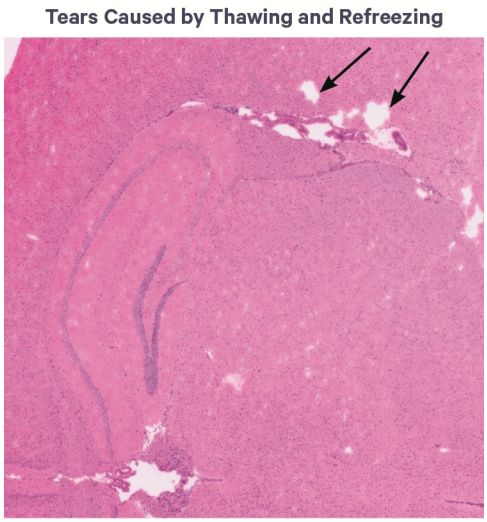
Perform H&E staining as described in 2.6 H&E Staining on page 47, coverslip as described in 2.7 Coverslip Mounting on page 49 and image as described in 2.8 Imaging on page 50.

Quality Assessment

Ensure that the tissue section is free from tissue processing and sectioning artifacts as shown in the images below and in Troubleshooting on page 51. Tissue processing artifacts may include improper snap freezing, squeeze/crush artifacts, ice crystal artifacts, and hemorrhaging. If imaging reveals satisfactory tissue morphology, proceed with the appropriate staining protocol using a different tissue slide.







Staining protocol:

- 2. H&E Staining & Imaging on page 33

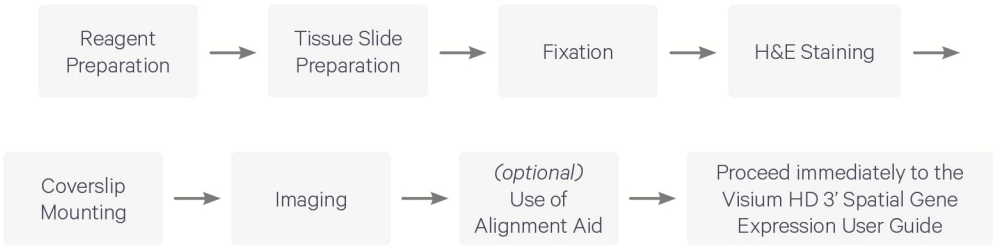
2. H&E Staining & Imaging

2.0 Overview

This chapter provides guidance on the fixation, H&E staining, and imaging of fresh frozen tissue slides. Process up to two tissue slides at a time. Ensure that microscope settings have been verified and imaging programs have been created prior to starting this protocol. Consult the Imaging System Requirements & Recommendations section of the Visium HD Spatial Applications Imaging Guidelines Technical Note (CG000688) for more information.

Failure to complete protocol steps quickly may result in RNA degradation.

Overview of H&E Staining & Imaging



2.1 Get Started

Consult the Visium HD 3' Spatial Gene Expression Protocol Planner (CG000803) for a list of third-party items. If using containers other than slide mailers, scale volumes appropriately so that tissue sections are completely covered by reagent.

Unless otherwise indicated, the preparation steps described in this section are for processing up to two tissue slides at a time.

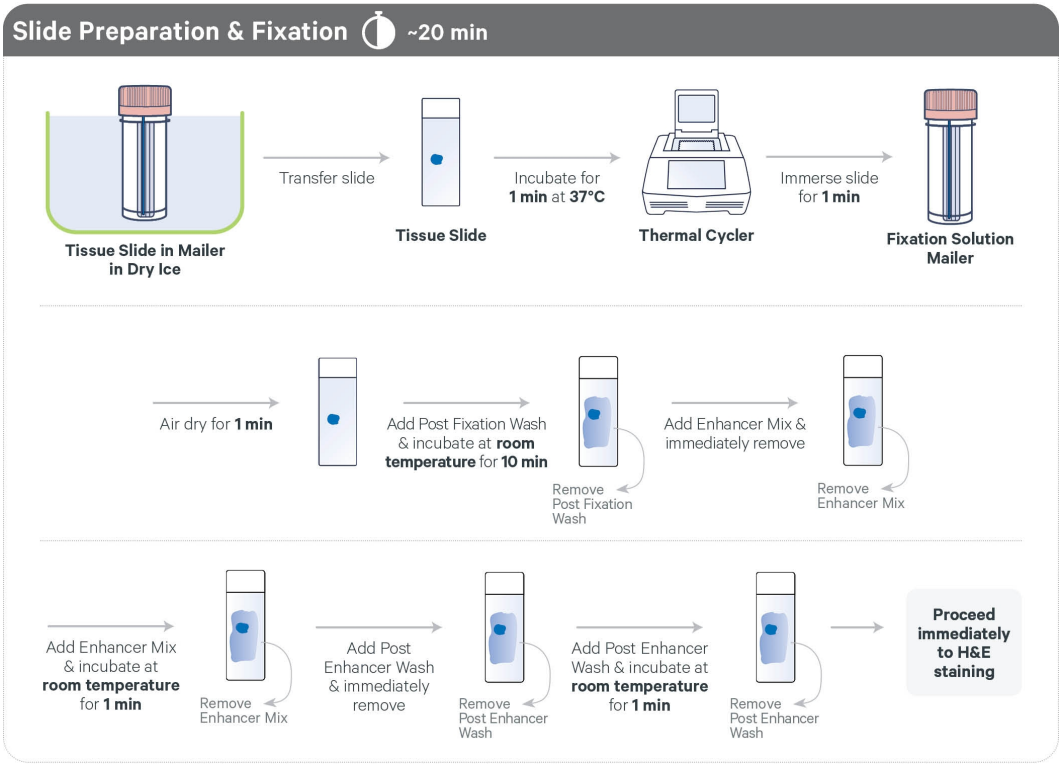
Ensure that all solutions are prepared prior to removing tissue slides from **-80°C**.

For Fixation & Permeabilization

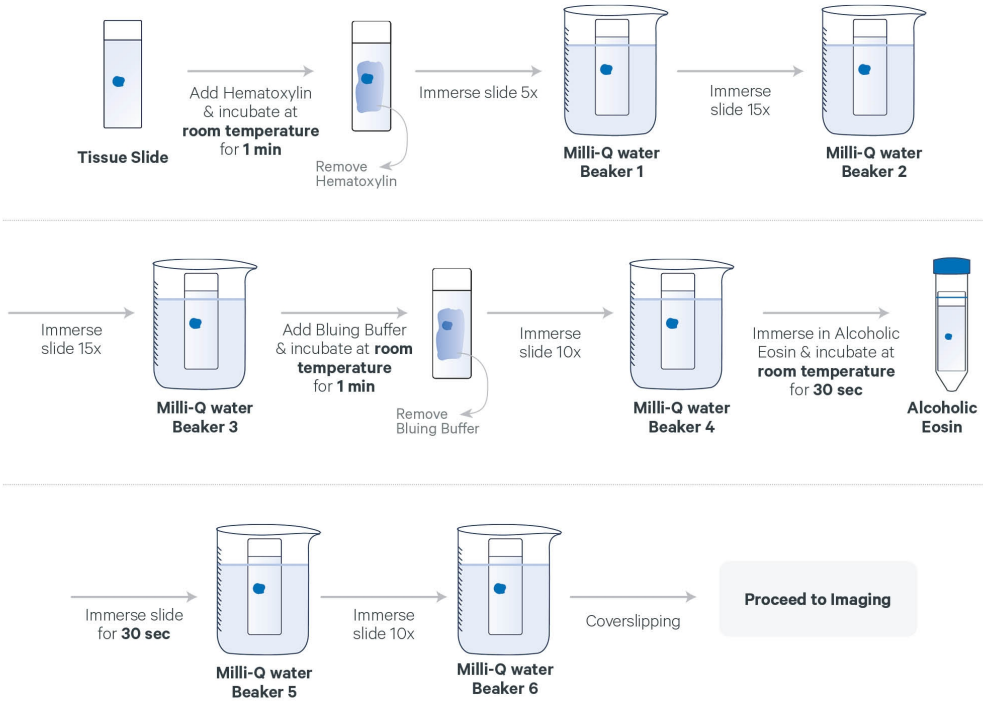
Items			Preparation & Handling	Storage
Maintain at Room Temperature				
☐ ◯ Reducing Agent B	2000087	Thaw at room temperature, vortex, verify no precipitate, centrifuge briefly.	-20°C
Maintain on Ice				
☐ ● RNase Inhibitor B	2001400	Thaw on ice, vortex, verify no precipitate, centrifuge briefly.	-20°C
Obtain				
☐ Nuclease-free Water	-	-	Ambient
☐ PBS	-	Either 1X PBS or 10X PBS may be used. If using 10X PBS stock, PBS must be diluted to 1X as indicated in the Reagent Preparation section.	Ambient
☐ Acetone	-	-	Ambient
☐ Methanol	-	-	Ambient
☐ Alcoholic Eosin	-	-	Ambient
☐ Gill II Hematoxylin	-	-	Ambient
☐ Bluing Buffer	-	-	Ambient
☐ Slide Mailers	-	-	Ambient
☐ Forceps	-	-	Ambient
☐ 65°C Temperature-controlled Instrument	-	Appropriate instruments include bead baths, heat blocks, and thermomixers. Instrument should be able to accommodate 1.5-ml or 2-ml centrifuge tubes.	Ambient

Items			Preparation & Handling	Storage
☐	37°C Temperature-controlled Instrument	-	Appropriate instruments include bead baths, heat blocks, and thermomixers. Instrument should be able to accommodate 1.5 or 2-ml centrifuge tubes.	Ambient
☐	25 ml Reagent Reservoir	-	-	Ambient

2.2 Protocol Overview



H&E Staining & Coverslipping ~15 min



Wipe excess liquid from the back of the slide after each immersion series

2.3 Reagent Preparation

Prepare the following before starting the Visium HD 3' H&E staining workflow. Buffers should be prepared fresh. If using slide containers other than a 12 ml slide mailer, reagent volumes will need to be scaled appropriately so that tissue sections are completely covered by reagent.

a. Clean workspace, pipettes, and gloves with an RNase decontamination solution, followed by 70% ethanol.

b. Place Low Profile Thermocycler Adapter in thermal cycler set to incubate at **37°C.** DO NOT close lid.

c. Pre-heat appropriate temperature-controlled instrument to **37°C.**

d. Pre-heat appropriate temperature-controlled instrument equipment to **65°C.**

e. Prepare Fixation Solution according to the table below, adding reagents in the order listed. Invert gently to mix. Fixation Solution may be prepared up to 24 h in advance and stored at –20°C. This volume of Fixation Solution is sufficient for processing two tissue slides.

Fixation Solution	Stock	Final	Total Amount (ml)
Methanol	100%	50%	5.0
Acetone	100%	50%	5.0
Total	**-**	**-**	**10.0**

f. Dispense **10 ml** Fixation Solution into mailer and label as Fixation Solution Mailer.

g. Move Fixation Solution Mailer to **-20°C**. Do not remove Fixation Solution Mailer from -20°C until Tissue Slide Preparation step is completed.

h. Clean seven 1L beakers:

- Spray with an RNase decontamination solution and leave for **10 sec** to **1 min**.
- Spray with 70% isopropanol or 70% ethanol.
- Rinse with Milli-Q water.

i. Dispense **800 ml** of Milli-Q water into each beaker.

j. Prepare **10 ml** of Alcoholic Eosin in a slide mailer for each tissue slide.

k. Prepare Mounting Medium in a 2-ml tube, adding reagents in the order listed. If necessary, perform an intermediate dilution for Reducing Agent B to avoid pipetting small volumes (e.g. use 2 µl of a 1:10 dilution instead of 0.1 µl of stock). If starting from a different concentration of Glycerol, recalculate volumes of remaining reagents appropriately.

Use a **wide-bore** pipette tip when pipetting glycerol. Vortex to mix. Briefly centrifuge to remove bubbles. Inspect for bubbles. Continue centrifuging until no bubbles remain.

Mounting Medium *1 slide = 1 Tissue Slide*	Stock	Final	1 Slide (µl)	2 Slides +10% (µl)
Glycerol	100%	85%	85.0	187.0
Reducing Agent B	-	-	0.2	0.4
RNase Inhibitor B	-	-	10.0	22.0
Nuclease-free water	-	-	4.8	10.6
Total	**-**	**-**	**100.0**	**220.0**

l. Prepare 1X PBS according to the table below in two 50-ml centrifuge tubes for a total of 100 ml 1X PBS, adding reagents in the order listed. Vortex. Maintain at room temperature.

1X PBS	Stock	Final	Total Amount (ml)
Nuclease-free water	-	-	45.0
PBS	10X	1X	5.0
Total	**-**	**-**	**50.0**

m. Fill two 2-ml centrifuge tubes with 1X PBS and warm at **37°C** for **10 min**. Maintain at 37°C.

n. Prepare Post Fixation Wash in a 15-ml centrifuge tube according to the table below, adding reagents in the order listed. Vortex. Maintain at room temperature.

Label 15-ml centrifuge tube tube as Tube 1.

	Post Fixation Wash	Stock	Final	1 Slide (µl)	2 Slides + 5% (µl)
	1X PBS	-	-	1,320	2,772
○	Reducing Agent B	-	-	30	63
●	RNase Inhibitor B	-	-	150	315
	Total	**-**	**-**	**1,500**	**3,150**

o. Prepare Post Enhancer Wash in a 15-ml centrifuge tube according to the table below, adding reagents in the order listed. Vortex. Maintain at room temperature.

Label 15-ml centrifuge tube as Tube 3 (Tube 2 will be prepared later in the protocol).

	Post Enhancer Wash	Stock	Final	1 Slide (µl)	2 Slides + 5% (µl)
	1X PBS	-	-	1,796.0	3,771.6
○	Reducing Agent B	-	-	4.0	8.4
●	RNase Inhibitor B	-	-	200.0	420.0
	Total	**-**	**-**	**2,000.0**	**4,200.0**

p. Prepare a bucket of dry ice.

q. Prepare a bucket of ice.

r. Obtain Enhancer (either an aliquot or stock tube) and place on ice.

s. Follow the appropriate Enhancer thawing guidance.

If thawing Enhancer for the first time:

- Incubate Enhancer (PN-2000482) at **65°C** for **10 min** or until solution is reconstituted to a dark green solution with no visible particulates. DO NOT exceed 20 min.

Before Incubation (left) & After Incubation (right)

- Vortex briefly and aliquot into four 1.5-ml centrifuge tubes (430 µl per tube). If using an aliquot immediately, store at room temperature until use, gently inverting every 10 min. Immediately move remaining aliquots to ice, then store at -20°C. DO NOT exceed 2 freeze-thaw cycles per aliquot.

If using an Enhancer aliquot frozen at -20°C:

- Incubate aliquot at **65°C** for **10 min** or until solution is reconstituted to a dark green solution with no visible particulates. DO NOT exceed 20 min. Store at room temperature until use. Gently invert every 10 min.

t. Obtain reagent reservoir. The same reagent reservoir can be used throughout the protocol.

u. Retrieve Fixation Solution Mailer from **-20°C** and place next to thermocycler on ice.

v. Remove slide mailer containing stored fresh frozen tissue slides from **-80°C** and bury into the dry ice.

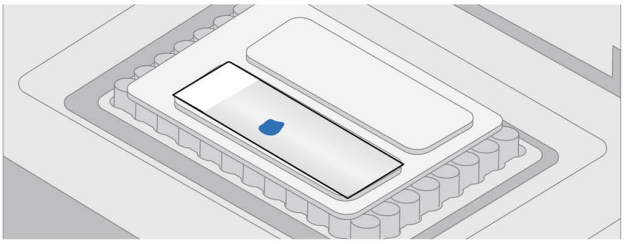
Alternatively, submerge an uncapped empty slide mailer in dry ice and incubate for **5 min**. *Remove slides from* **-80°C** *storage with forceps and immediately place in pre-chilled empty slide mailer on dry ice.*

2.4 Tissue Slide Preparation

a. Move slides from dry ice to the Thermocycler Adapter with tissue side facing up on a **37°C** pre-heated thermal cycler. Ensure Sample Area is aligned with the corresponding area on the Thermocycler Adapter.

b. Incubate for **1 min**. DO NOT close lid.

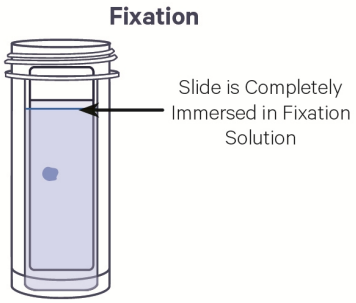
Incubation on a Thermal Cycler

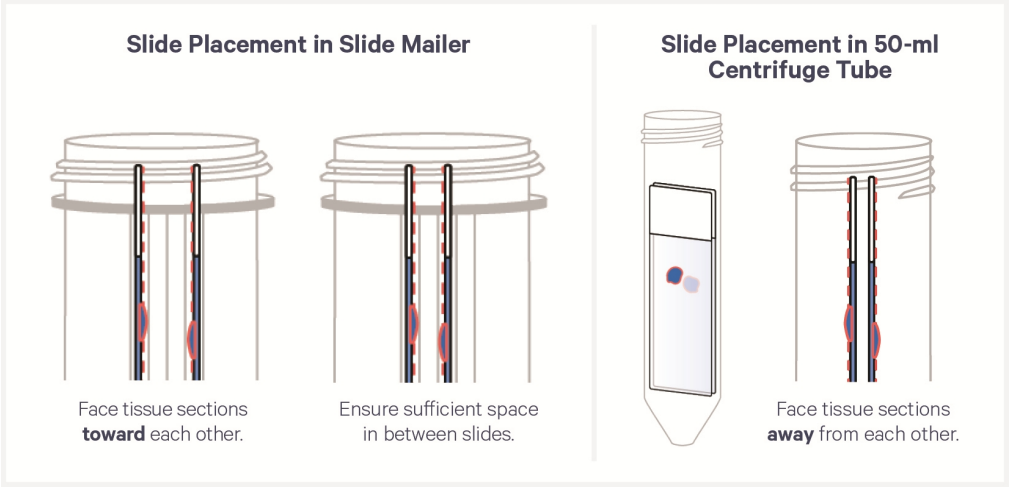


2.5 Fixation

To avoid disturbing tissue, pipette at least 1 cm away from the tissue edge and avoid pipetting at an angle.

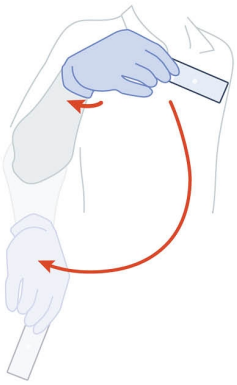
a. **Immediately** remove slide from thermal cycler following incubation. Gently immerse slide in Fixation Solution Mailer on ice and incubate for **1 min**. Follow slide placement mailer guidance in the illustration below for all steps where a slide is placed in a slide mailer or centrifuge tube.





b. Remove slide from fixative, flick, and remove excess fixative from the back of the slide with a lint-free laboratory wipe.

DO NOT touch the tissue sections on the slide.

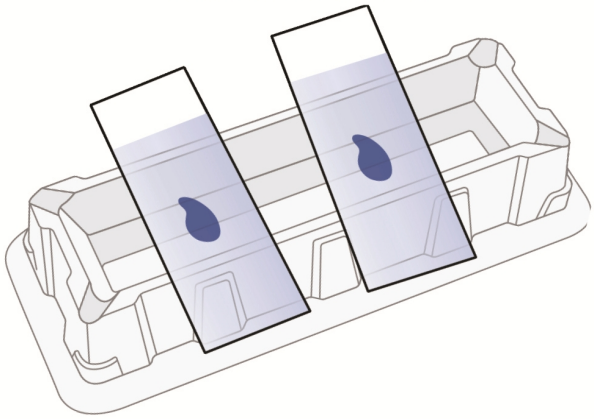


Flick the Slide

Lead with the elbow and allow the wrist to rotate

c. Place slides over reagent reservoir with tissue sections facing up and air dry for **1 min**. DO NOT place more than two tissue slides over a reagent reservoir.

Place Slides Over Reservoir



d. Add **750 µl** from Tube 1 (Post Fixation Wash) to each tissue section, followed by an additional **750 µl** from Tube 1 to each tissue section for a total of **1.5 ml** of Post Fixation Wash per tissue section.

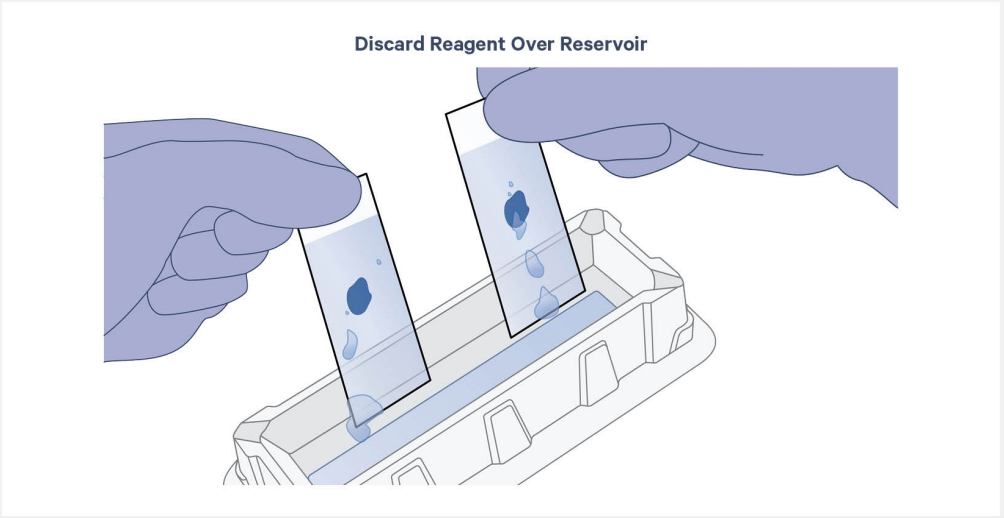
e. Incubate at **room temperature** for **10 min**.

f. Before Post Fixation Wash incubation is complete, prepare Enhancer Mix as described below in a 15-ml centrifuge tube. Vortex. Maintain at 37°C.

Label 15-ml centrifuge tube as Tube 2.

Enhancer Mix	Stock	Final	1 Slide (µl)	2 Slides + 5% (µl)
Pre-warmed 1X PBS	-	-	1,800	3,780
Enhancer	-	-	200	420
Total	**-**	**-**	**2,000**	**4,200**

g. Remove Post Fixation Wash by tilting slides over the reagent reservoir. DO NOT allow slides to touch liquid remaining in the reservoir.



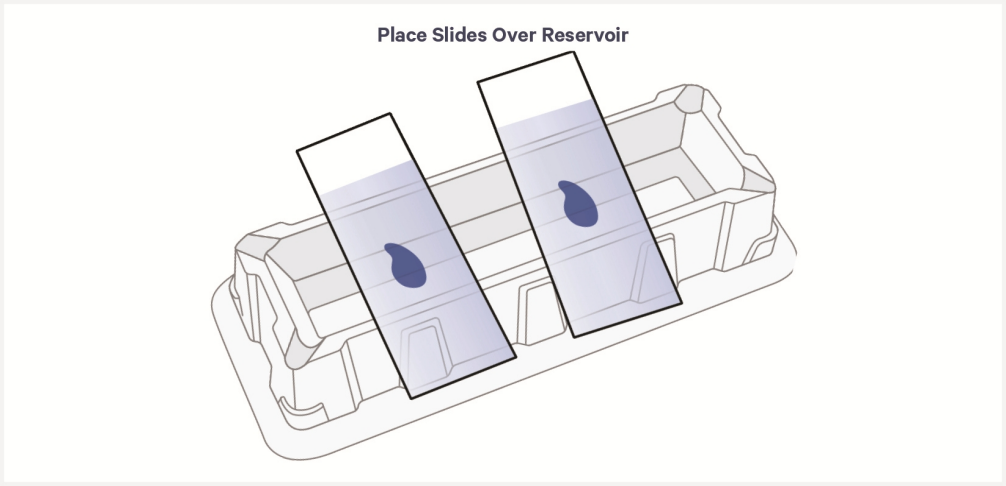
h. Place slides with tissue sections facing up over the reagent reservoir.

DO NOT allow slides to dry.

i. Add **1 ml** from Tube 2 (Enhancer Mix) to uniformly cover tissue sections.

j. **Immediately** remove Enhancer Mix by tilting slides over the reagent reservoir.

k. Place slides with tissue sections facing up over the reagent reservoir.



l. Add **1 ml** from Tube 2 (Enhancer Mix) to uniformly cover tissue sections.

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

n. Remove Enhancer Mix by tilting slides over the reagent reservoir.

o. Place slides over the reagent reservoir.

DO NOT allow slides to dry.

p. Add **1 ml** from Tube 3 (Post Enhancer Wash) to uniformly cover tissue sections.

q. **Immediately** remove Post Enhancer Wash by tilting slides over the reagent reservoir.

r. Place slides over the reagent reservoir.

s. Add **1 ml** from Tube 3 (Post Enhancer Wash) to uniformly cover tissue sections.

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

u. Remove Post Enhancer Wash by tilting slides over the reagent reservoir.

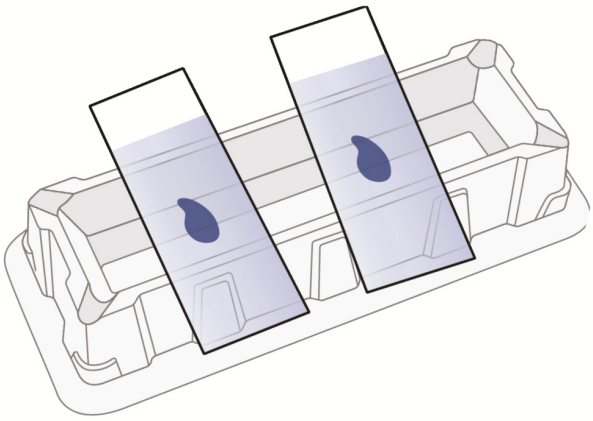
v. Proceed immediately to 2.6 H&E Staining on the next page.

2.6 H&E Staining

To avoid disturbing tissue, pipette at least 1 cm away from the tissue edge and avoid pipetting at an angle.

a. Place slides over reagent reservoir with tissue sections facing up. DO NOT place more than two tissue slides over a reagent reservoir.

Place Slides Over Reservoir

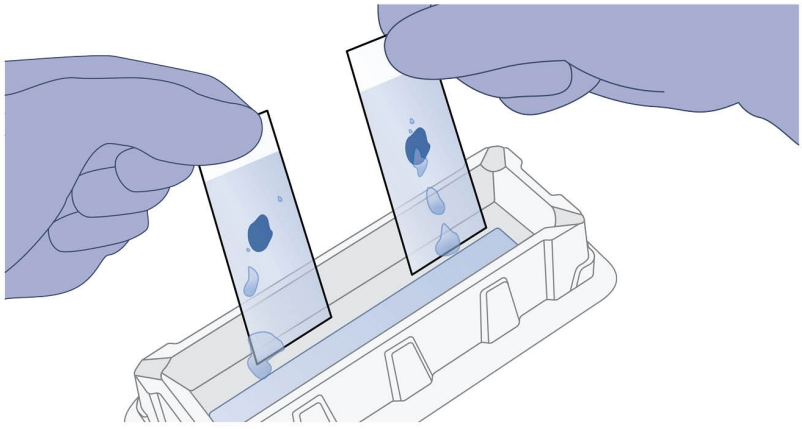


b. Add **1 ml** Hematoxylin per slide to uniformly cover all tissue sections.

c. Incubate **1 min** at **room temperature**.

d. Discard reagent by holding slides at an angle over a reagent reservoir.

Discard Reagent Over Reservoir



e. Immerse slides 5x in Water Beaker 1.

f. Immerse slides 15x in Water Beaker 2.

g. Immerse slides 15x in Water Beaker 3.

h. Wipe excess liquid from back of slides with a lint-free laboratory wipe without touching the tissue section.

i. Place slides over reagent reservoir with tissue sections facing up.

j. Add **1 ml** Bluing Buffer per slide to uniformly cover all tissue sections.

k. Incubate **1 min** at **room temperature**.

l. Discard reagent by holding slides at an angle over a reagent reservoir.

m. Immerse slides 10x in Water Beaker 4.

n. Wipe excess liquid from back of slides with a lint-free laboratory wipe without touching the tissue section.

o. Gently immerse slides in alcoholic Eosin solution in separate slide mailers. DO NOT use diluted Eosin.

p. Incubate **30 sec** at **room temperature**.

q. Discard reagent by holding slides at an angle with the bottom edge in contact with a lint-free laboratory wipe.

r. Immerse slides for **30 sec** in Water Beaker 5.

s. Immerse slides 10x in Water Beaker 6.

t. Wipe excess liquid from back of slides with a lint-free laboratory wipe without touching the tissue section.

u. Immediately proceed to 2.7 Coverslip Mounting on the next page.

DO NOT air dry the slides.

2.7 Coverslip Mounting

a. Gently touch the long edge of the slide on a lint-free laboratory wipe to remove excess moisture. Ensure any large droplets are removed.

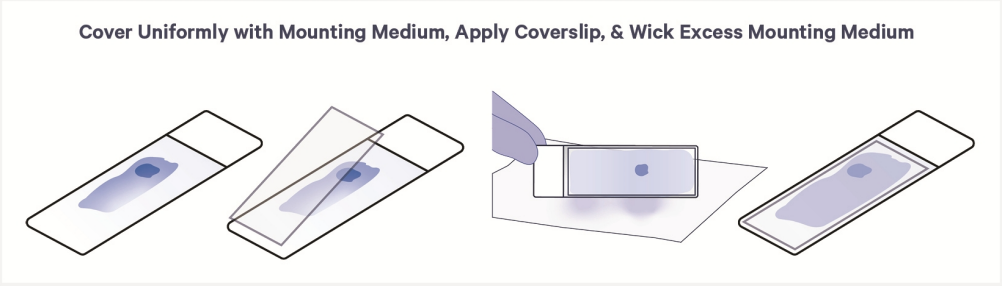
b. Place slides on a flat, clean, nonabsorbent work surface. Some residual droplets may remain.

DO NOT allow slides to dry.

c. Using a **wide-bore** pipette tip, add **100 µl** Mounting Medium to cover the entire tissue section.

DO NOT use Cytoseal or nail polish for securing the coverslip.

d. Slowly apply coverslip at an angle on one end of the slides, without introducing bubbles. Allow Mounting Medium to spread and settle.



e. Remove any large excess of Mounting Medium by carefully wicking away from the edge of the coverslip using a lint-free laboratory wipe. Do not move coverslip and disturb tissue.

f. **Immediately** proceed with imaging or store slides laying flat in a slide holder. Ensure that slides are laid flat to prevent loss of Mounting Medium. Store slides in the dark at **4°C** for up to **24 h**.

2.8 Imaging

a. If stopping point was used, remove tissue slides from 4°C and remove condensation on the back of the tissue slides with a lint-free laboratory wipe.

b. Image tissue section of interest at desired magnification using brightfield imaging settings. Consult the Visium HD Spatial Applications Imaging Guidelines Technical Note (CG000688) for additional information.

c. If using the Visium CytAssist Alignment Aid to add annotations to the tissue slide, do so while the tissue slide is coverslipped and before moving to the User Guide. For more information, consult Visium CytAssist Alignment Aid on page 11.

d. Lay slides flat in a slide holder, place in the dark at **4°C**, and proceed **immediately** to the Visium HD 3' Spatial Gene Expression User Guide (CG000805). Slides should remain at **4°C** until the Coverslip Removal & Destaining step in the user guide.

If not proceeding immediately, store slides laying flat in a slide holder in the dark at **4°C** for up to **72 h** prior to proceeding to the user guide. Ensure that slides are laid flat to prevent loss of Mounting Medium.

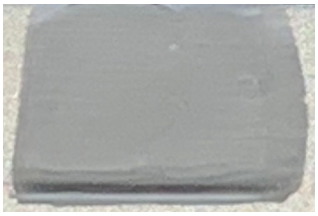
DO NOT let the attached coverslip dry out.

Troubleshooting

Tissue Preparation Troubleshooting

Cryosectioning - Impact of Cryostat Specimen Head Temperatures on Tissue Tearing

-10°C



Normal Section

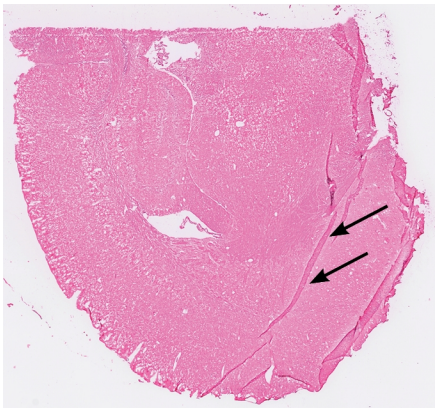
-14°C

-20°C

-30°C

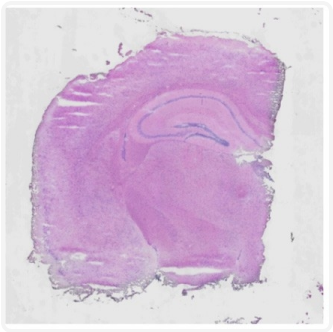
Tissue section has significant tearing.

Cryosectioning - Impact of Warm Tissue Block on Cryosectioning

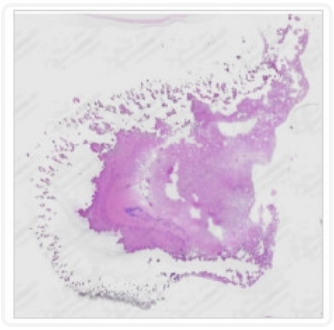


Folds in tissue are a result of warmer than optimal sectioning temperature.

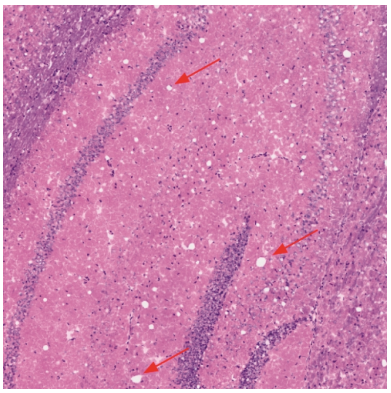
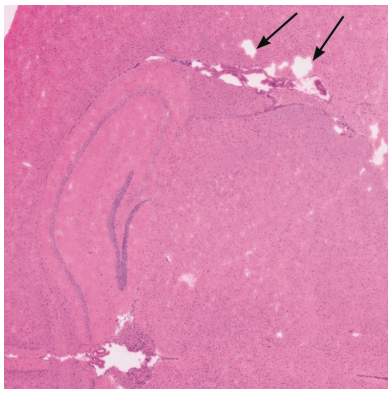
Section Placement - Impact of Condensation on Tissue Sections

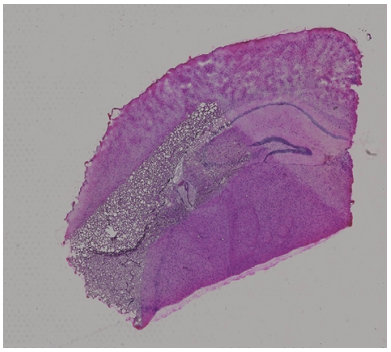
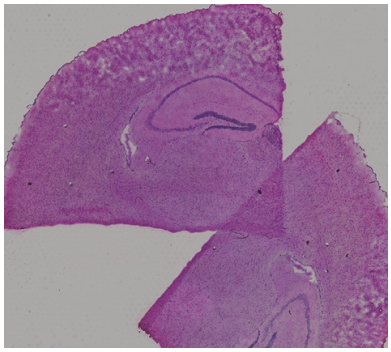


No Condensation. Intact tissue section.



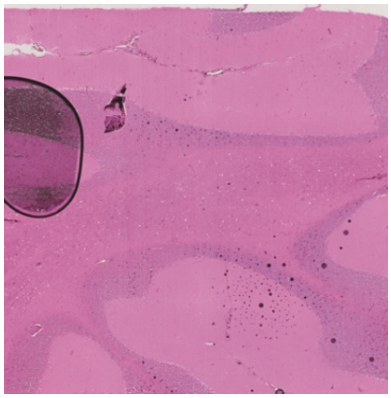
Tissue degraded due to condensation. DO NOT leave slides at room temperature, especially with fresh sections as the resulting condensation will cause tissue disintegration.

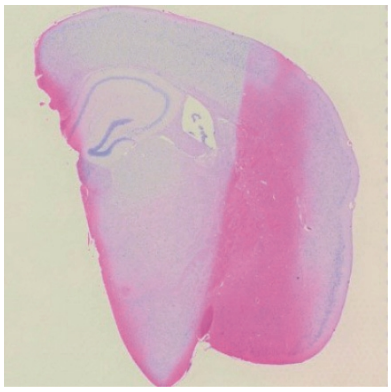
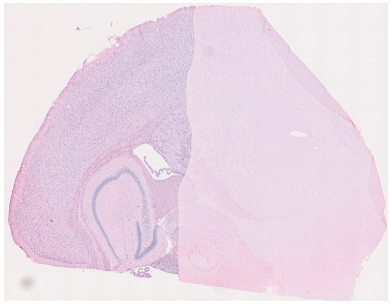
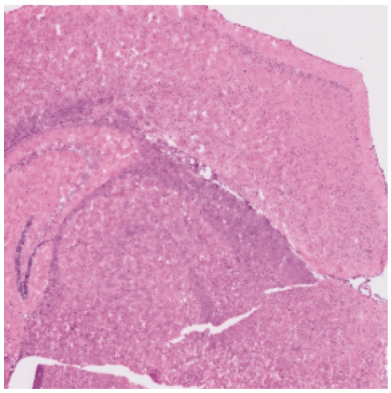
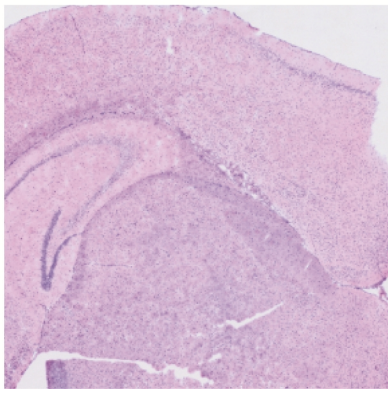
Impact of Freeze/Thaw Cycles on Tissue Slide or Failure to Thaw Tissue Slide	
	
Shortened thaw during tissue preparation causes small holes	Thawing and refreezing causes severe artifacts

Section Placement - Incorrect Placement of Tissue Sections	
	
Folded tissue section	Overlapping sections

Practice correct section placement on blank slides.

H&E Staining Troubleshooting	
Bubbles	Avoid bubble formation during coverslip mounting. Bubbles can be mitigated by applying the coverslip at an angle and slowly lowering it onto the slide, allowing air to escape. Centrifuge mounting medium to remove bubbles before use. Avoid introducing bubbles when pipetting mounting medium onto slide.



H&E Staining Troubleshooting	
Uneven Staining	**Causes** Ensure fresh, unexpired staining reagents are applied to the tissue uniformly and adequate washes are performed. A gentle tap may help reagents spread uniformly. Consider filtering hematoxylin.  Uneven staining may also be caused by allowing slides to dry during staining. Ensure that slides do not air dry prior to coverslip mounting. 
Incorrect Staining Protocol	The staining protocol in this handbook was optimized for tissues that will be processed with the Visium HD 3' assay. Using an alternative H&E staining protocol may result in reduced staining performance. Additionally, using staining dyes that are not listed in the Visium HD 3' Protocol Planner (CG000803) may require additional optimization and result in variable performance. **Alcoholic Eosin** **Aqueous Eosin**  

Notices

Document Number

CG000804 | Rev A

Legal Notices

Instrument & Licensed Software Updates Warranties

Updates to existing Instruments and Licensed Software may be required to enable customers to use new or existing products.

Support

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Document Revision Summary

Document Number

CG000804

Title

Visium HD 3' Spatial Gene Expression Handbook

Revision

Rev A

Revision Date

June 9, 2025

Description of Changes

Take 1 minute to evaluate this protocol. Scan this code or click here.