

Atera Gene Expression – Fresh Frozen Tissue Preparation Handbook

Introduction

The Atera Gene Expression workflow is designed to measure mRNA in tissue sections derived from fresh frozen (FF) and embedded tissue samples and requires a slide with intact tissue sections as input. Proper tissue handling, storage, and preparation techniques preserve the morphological quality of tissue sections and integrity of mRNA transcripts.

This FF Tissue Handbook provides guidance on:

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

Additional Guidance

Consult the 10x Genomics Support website for additional resources.

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Document Revision Summary

Handbook Overview and Navigation

Overview

This Handbook describes Fresh Frozen tissue and sample preparation for the Atera Gene Expression workflow. Tabs on the right-hand side of the page denote different sections of this Handbook.

Atera Gene Expression Reagent Kits

Atera Fresh Frozen Sample Prep Reagents (2 rxns) PN-1001113

Consult SDS for handling and disposal information.

Atera Fresh Frozen Sample Prep Reagents (2 rxns) PN-1001113 <i>Shipped on dry ice; Store at -20°C</i>					
Cap Color	Reagent	PN	Cap Color	Reagent	PN
●	Atera Fixation Additive	2001977	○	Additive A*	220093
●	Atera Wash Enhancer L	2001978	○	Atera Hyb Buffer**	2001879



*Additive A is also used in the Atera Gene Expression User Guide. DO NOT discard reagent after using during the sample preparation workflow.

**Used in the Atera Gene Expression User Guide. Reagent should be thawed during this sample preparation workflow when indicated.

Atera Cassette Kit (2 cassettes) PN-1001153

Atera Cassette Kit PN-1001153 <i>Shipped at ambient temp Store at ambient temp</i>	
	#
Atera Cassette Top	2
Atera Cassette Bottom	2
Atera Cassette Insert	4
Atera Cassette Lid	2

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Third-Party Items

Successful execution of this 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 before starting this workflow.

Consult the Atera Gene Expression Protocol Planner (CG001669) for a list of the following third-party items:

- Additional reagents, kits, and equipment
- 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



Indicates a video is available for the specified technique

Videos

- Videos describing key steps in the protocol can be found [here](#).
- When a relevant video is available for a protocol step, it will be marked with a video icon.

General Reagent Handling

- Fully thaw reagents at indicated temperatures. Thoroughly mix reagents before use.
- When pipette mixing reagents, unless otherwise specified, set pipette to 75% of total volume.
- Promptly move reagents back to the recommended storage.
- In reagent preparation tables:
 - 1X, 2X, 4X refer to the number of slides/cassettes unless otherwise specified.
 - Volumes may not scale linearly as the number of reactions increases.

Pipette Calibration

Follow manufacturer's calibration and maintenance schedules.

Tissue Scoring

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

Cryosectioning Temperature

- Cryosectioning temperature settings depend upon local conditions, tissue types, and the cryostat used. They should be optimized based on the quality of resulting tissue sections.
- If optimal sectioning temperature is unknown, begin with -20°C for blade and -10°C for the specimen head and adjust accordingly.
- During prolonged sectioning periods, allow the cryostat temperature to equilibrate by briefly closing the chamber.

Sectioning Speed

- Sectioning speed is dependent on the tissue composition. Challenging tissues (fatty tissues like breast as well as bone, cartilage, etc.) typically require slower sectioning speed.
- Faster sectioning speed may crack or tear the sections or damage the tissue block or cryostat.

Section Thickness

- Recommended section thickness is 10 μm .

Slide Handling

- Always wear gloves when handling slides.
- The tissue sections should always be placed facing up on labeled-side of the slide.
- Hold the slide on the label. DO NOT touch the tissue sections on the slide.
- Minimize exposure of the slides to sources of particles and fibers.
- Keep the slide flat on the bench when adding reagents to the Sample Area.
- Ensure that no absorbent surface is in contact with the reagents on the slide during incubation.
- When pipetting reagent onto a slide, avoid generating bubbles.
- Applying excessive force to the slide may cause the slide to break.

Section Placement

- Discard the first couple of sections after facing the block before section placement.
- Place the tissue section within the Sample Area on the slides.
 - For information on determining the Sample Area on a slide, consult the Atera Sample Placement Quick Reference Card (CG001675).

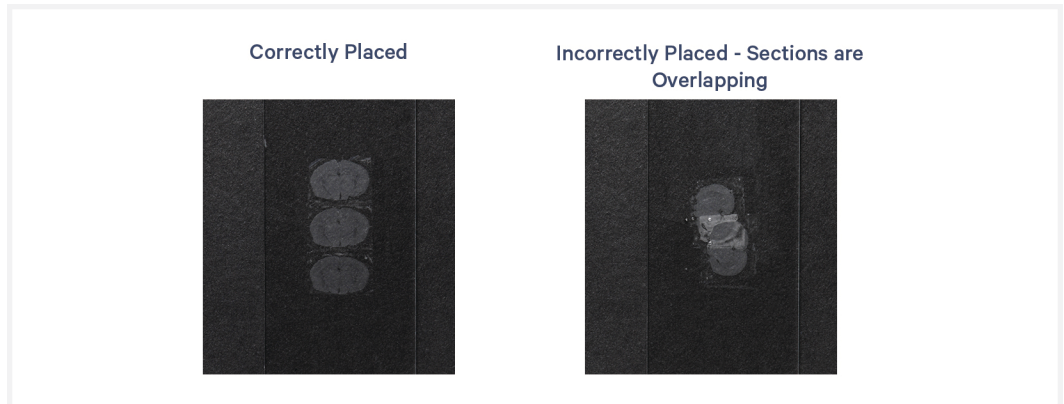


- The section on the slides should be uniform without any cracks, tears, or folds.
- After section placement, sections cannot be repositioned as this would compromise assay performance.
- 10x Genomics recommends using the tissue alignment tool to ensure tissue is placed within the Sample Area.
- Tissue outside the Sample Area will not be analyzed and may be damaged during the instrument run.
- Tissue underneath the gasket during cassette assembly does not require removal. DO NOT attempt to remove this tissue, which may cause tissue detachment.

Placing Multiple Sections

- Place sections at least 1 mm apart from one another to maximize the likelihood each can be selected as its own region during the instrument run. For more information on region selection, see the Atera Instrument User Guide (CG001673).

- The OCT surrounding each tissue section should not overlap with the OCT or tissue of an adjacent section.



Tissue Detachment

- Monitor section adhesion on slides throughout the workflow.
- Tissue detachment during the workflow can negatively impact performance.
- For more information, see [Troubleshooting](#).

Slide Storage

- Store the sealed container containing slides with fresh frozen tissue at -80°C for up to 4 weeks.

Atera Cassette

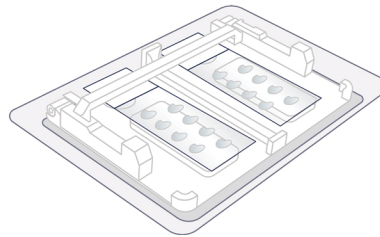
- Consult the Atera Cassette Assembly, Disassembly, and Use Quick Reference Card (CG001674) for information on:
 - Cassette assembly and removal
 - Reagent addition and removal
 - Atera Cassette Lid application and removal

Slide Incubation

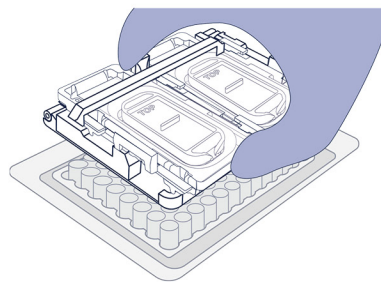
Incubation on a Thermal Cycler

- Position an Atera Thermocycler Adaptor on a thermal cycler that is set at the incubation temperature.
 - Ensure thermal cycler has reached appropriate temperature before starting incubation.
 - Ensure that the Atera Thermocycler Adaptor is in contact with the thermal cycler surface uniformly.
- Place the slide on the Atera Thermocycler Adaptor with the tissue facing up.
 - Ensure that the entire bottom surface of the slide is in contact with Atera Thermocycler Adaptor.

DO NOT close the thermal cycler lid when incubating the slide due to the risk of touching the tissue.

Slides on Thermocycler Adaptor

- When incubating a slide encased in an Atera Cassette, place the assembled unit on the Thermocycler Adaptor with the well facing up.
- The cassette should always be sealed with an Atera Cassette Lid when on the Thermocycler Adaptor unless indicated otherwise.

Atera Cassettes on Thermocycler Adaptor

Hold the adaptor at the center with the thumb and index finger, as illustrated

Incubation at Room Temperature

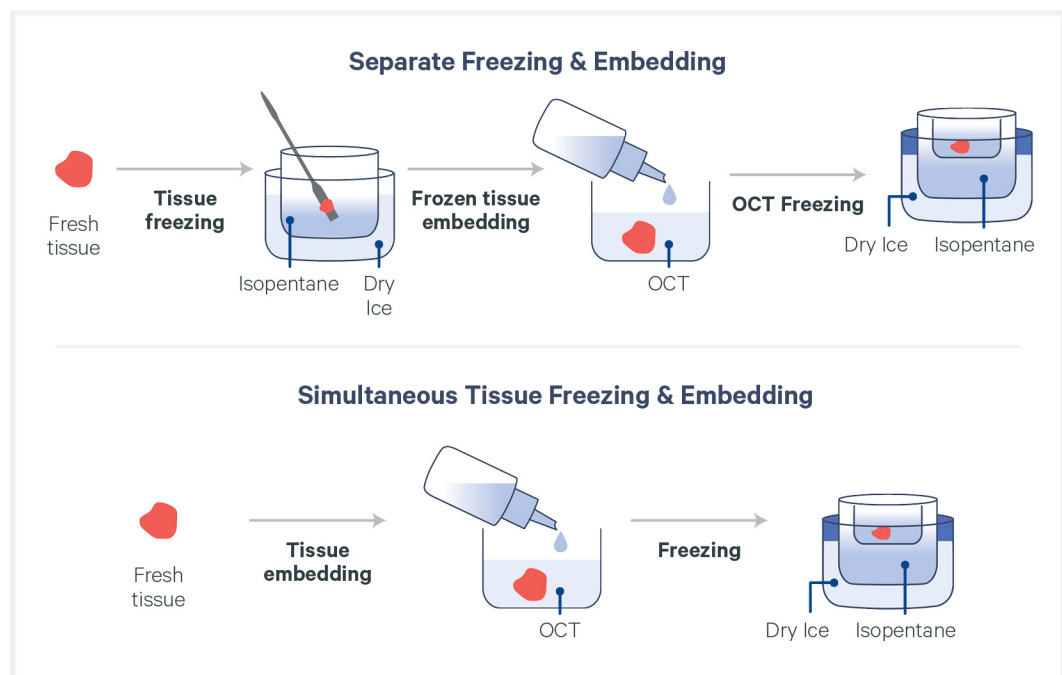
- Place slides with label toward the top of the staining container (mailer or staining rack in staining dish) for incubations at room temperature.
- Separate multiple slides by at least one slotted channel.
- Avoid placing slides in the first or last slotted channel of the mailer or slide staining rack. Slides with tissues in these positions may get scratched.
- Ensure mailers are in an upright position during incubation.

1. Tissue Freezing & Embedding

Overview

This section provides guidance on tissue freezing and embedding. Freshly obtained tissue samples must be snap frozen to prevent RNA degradation and avoid crystal formation, which can lead to morphological damage to the tissue. Once frozen, tissue samples are embedded in a freezing and embedding compound, Optimal Cutting Temperature (OCT), to preserve the structure of the tissue and to provide structural support during cryosectioning. If performing separate freezing and embedding, see [Separate Tissue Freezing and Embedding on page 17](#).

Alternatively, perform simultaneous freezing and embedding in OCT for tissues with crevices/gaps or tissues that have a tendency to curl. First, fresh tissues are embedded in OCT. Then, the cryomold is lowered into isopentane to freeze both tissue and OCT. If performing simultaneous freezing and embedding, see [Simultaneous Tissue Freezing & Embedding on page 16](#).



Tissue Freezing

Tissue should be snap frozen in isopentane (separate freezing and embedding) or embedded in OCT prior to freezing (simultaneous freezing and embedding).

Isopentane should be chilled for at least 30 min prior to freezing and monitored for evaporation.

Tissue Embedding

Before cryosectioning, either fresh or frozen tissue samples are embedded in OCT. Assuming proper tissue spacing and rapid OCT freezing, multiple tissues can be placed within the same cryomold. Unlike paraffin or resin-based embedding techniques, OCT does not interact with proteins and other molecules that impact antigenicity.

OCT embedding of the tissue offers the following advantages:

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

1.0 Simultaneous Tissue Freezing & Embedding

Items	Preparation & Handling
Prepare	
Isopentane beaker	In a fume hood, pour 200 ml isopentane into a polypropylene beaker and place the beaker on dry ice for ≥ 30 min.
OCT	Place OCT on ice for ≥ 30 min.
Tissue	Using a rolled up laboratory wipe, absorb excess moisture from the surface of the tissue to limit ice crystal formation.

- Label the cryomold to mark the orientation of the tissue.
- Add a few drops of room temperature OCT into the cryomold.
- Using forceps, transfer tissue into the cryomold with OCT. If necessary, adjust tissue orientation and add additional OCT to ensure tissue is fully covered. Confirm there are no bubbles, especially near the tissue. Let sit for **20 sec.**
- Using forceps, lower the cryomold containing embedded tissue into the isopentane without fully submerging. Keep cryomold in contact with isopentane until the OCT has solidified and turned white.
- Once frozen, place the cryomold on a piece of aluminum foil on dry ice for **30 min.**



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



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

1.1 Separate Tissue Freezing

Items	Preparation & Handling
Prepare	
Isopentane beaker	In a fume hood, pour 200 ml isopentane into a polypropylene beaker and place the beaker on dry ice for ≥ 30 min.
Tissue	Using a rolled up laboratory wipe, absorb excess moisture from the surface of the tissue to limit ice crystal formation.

- a. Using forceps, transfer the tissue onto a piece of aluminum foil.
- b. Wrap the tissue in aluminum foil, ensuring the tissue is completely wrapped.
- c. Using forceps, lower the wrapped tissue into the isopentane.
 - Freezing time depends on tissue size and composition. For a 10 × 10 mm tissue, freezing time is **~1 min.**
 - Avoid long freezing times as they can lead to morphological changes.
- d. Once frozen, use chilled forceps to lift the wrapped tissue out of the isopentane and place on dry ice.
- e. Use chilled forceps to unwrap the foil and transfer tissue to a chilled and airtight sealed container or resealable bag.
- f. Store frozen tissue at **-80°C** for **long-term** storage or immediately proceed to the next step (Frozen Tissue Embedding).



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

1.2 Frozen Tissue Embedding

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

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

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



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 chilled OCT without introducing bubbles.
- c. Place the cryomold on a piece of aluminum foil on dry ice.
- d. Using chilled forceps, place the frozen tissue into the OCT, covering any exposed surfaces with additional OCT and noting the tissue orientation. Confirm there are no bubbles, especially near the tissue.
- e. Using chilled forceps, immediately transfer the cryomold containing tissue and OCT to the isopentane for **1 min.**
- f. Transfer cryomold onto a piece of aluminum foil on dry ice for **30 min.**



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



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

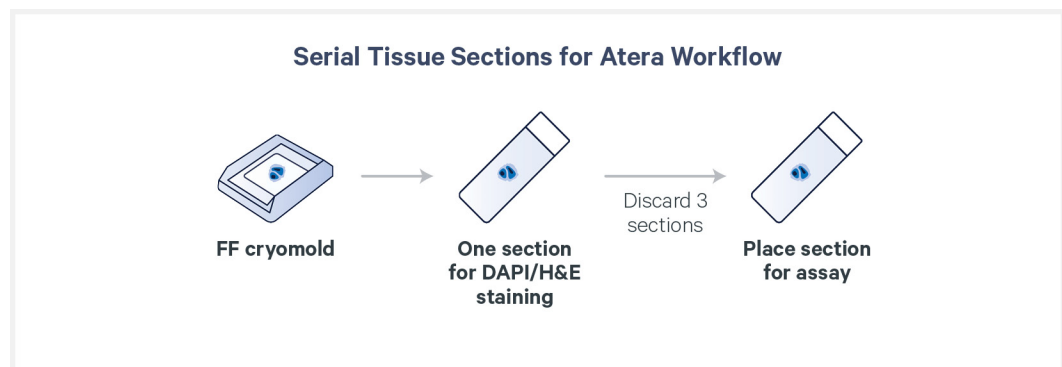
2. Cryosectioning & Quality Assessment

Overview

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



Quality assessment should be performed on a serial section that will NOT be used for the Atera assay.



Choose a Quality Assessment Method

Choose one or more quality assessment methods from the following options. Though quality assessment is optional, 10x Genomics strongly recommends performing DAPI and H&E quality assessment on one or more serial sections before starting the assay. Quality assessment is performed after sections are placed onto blank slides.

DAPI Staining

DAPI is a nuclear stain that enables assessment of nuclei quality, a predictor of assay success. However, good nuclei quality is not an absolute determinant of assay success.

H&E Staining

H&E staining allows for observation of tissue damage and artifacts that may result from poor tissue handling, sectioning, or fixation. These artifacts are correlated with poor assay outcomes. However, identification of artifacts may require histology expertise. Additionally, blood in the H&E image may result in high autofluorescence. High autofluorescence does not necessarily correlate with poor assay outcomes.

Cryosectioning

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

Cryostat Chamber Specifications

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

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

Section Placement

The tissue section is placed on a blank slide.

Fixation & Quality Assessment

Tissue sections are fixed and stained to allow for inspection. First, tissue sections are stained with a DAPI solution. Perform DAPI staining according to any preferred protocol. Stained tissue sections are then coverslipped and imaged. Tissue sections with bright, clear nuclei are considered good quality and will likely generate good data. Next, the same tissue section has its coverslip removed and is stained with Hematoxylin and Eosin (H&E) solutions.

Perform H&E staining according to any preferred protocol. A fixation protocol and an H&E protocol are provided in the Appendix. Stained tissue sections are then coverslipped and ready to image. Proceed immediately to imaging following the H&E Staining workflow. Review the H&E image thoroughly to assess tissue quality. Inspect tissue morphology for tissue processing and sectioning artifacts that may contribute to poor assay performance. See [Optional H&E Quality Assessment on page 27](#) for more guidance. If quality is satisfactory, proceed with [Cryosectioning & Section Placement](#).

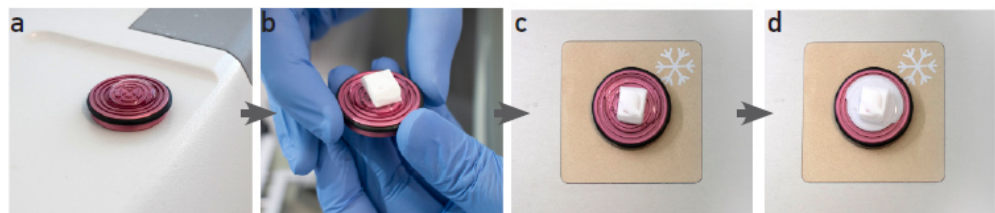
2.0 Get Started

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

2.1 Cryosectioning

Mount OCT Embedded Tissue Block on the Specimen Stage

- Fill the specimen stage (chuck) with OCT.
- Place the OCT embedded tissue block on the stage with the cutting surface facing away from the stage.
- Place the stage and the tissue block on the cryobar inside the cryostat chamber.
- Allow the OCT and the tissue block to freeze and adhere to the specimen stage for **5 min**.



Remove Excess OCT by Cryosectioning

- Once frozen, install the stage with the tissue block on to the specimen head of the cryostat and start sectioning to remove excess OCT.
- Sectioning conditions vary across different tissues and cryostats. Follow manufacturer's recommendation for cryosectioning.
- Continue sectioning until the tissue is visible.

Tissue Scoring

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

Tissue Scoring



2.2 Tissue Sectioning

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

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

If re-sectioning a used tissue block:

- For tissues prone to freeze/thaw damage, such as mouse brain, cut and discard ~7 sections with thickness set to 10 µm before collecting first section.
 - For other tissues, cut and discard ~5 sections with thickness set to 10 µm before collecting first section.
- b. Adhere tissue section to slide by gently touching the tissue section to the blank slide.
 - c. Immediately place a finger on the backside of the slide where the tissue section was placed to allow section to adhere to the slide.
 - d. Apply OCT to the exposed surface of the block and refreeze on cryobar before storage.

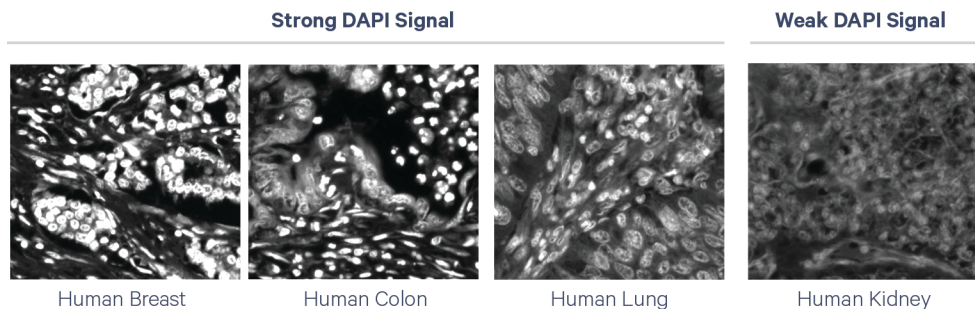
- e. Before proceeding to DAPI and H&E quality assessments, sections should be fixed. A fixation protocol is provided in the Appendix.
- f. After fixation, proceed to DAPI Staining.

2.3 Optional DAPI Quality Assessment

Review the DAPI image under the microscope at 20x magnification. Examine several areas within a single DAPI-stained section to ensure assessment is comprehensive. Tissue sections suitable for the assay should have:

- Punctate nuclei that are clearly defined
- DAPI staining that does not appear washed out

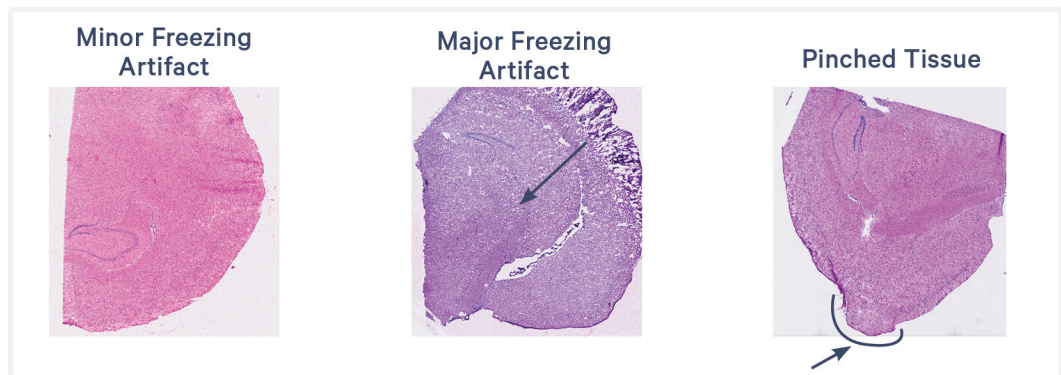
Sections with poor DAPI staining are unlikely to generate good data. All samples in the images below were imaged under the same conditions (405 nm channel).



2.4 Optional H&E Quality Assessment

Perform H&E staining according to any preferred protocol. An H&E protocol is provided in the Appendix. Review the H&E image thoroughly to assess tissue quality and select areas of interest. If scoring the block is necessary, see [Cryosectioning on page 23](#).

Ensure that the tissue section is free from tissue processing and sectioning artifacts as shown in the images below. Tissue processing artifacts may include improper snap freezing, squeeze/crush artifacts, ice crystal artifacts, and hemorrhaging. If imaging reveals satisfactory tissue morphology, proceed with [Cryosectioning & Section Placement](#).



3. Cryosectioning & Section Placement

Overview

This section provides guidance on tissue section placement. The resulting slide will be used for the Atera assay.

Cryosectioning

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

Section Placement

Before section placement, a morphology check is performed to confirm that the sections are free of artifacts. Sections are then placed within the Sample Area on the slide.

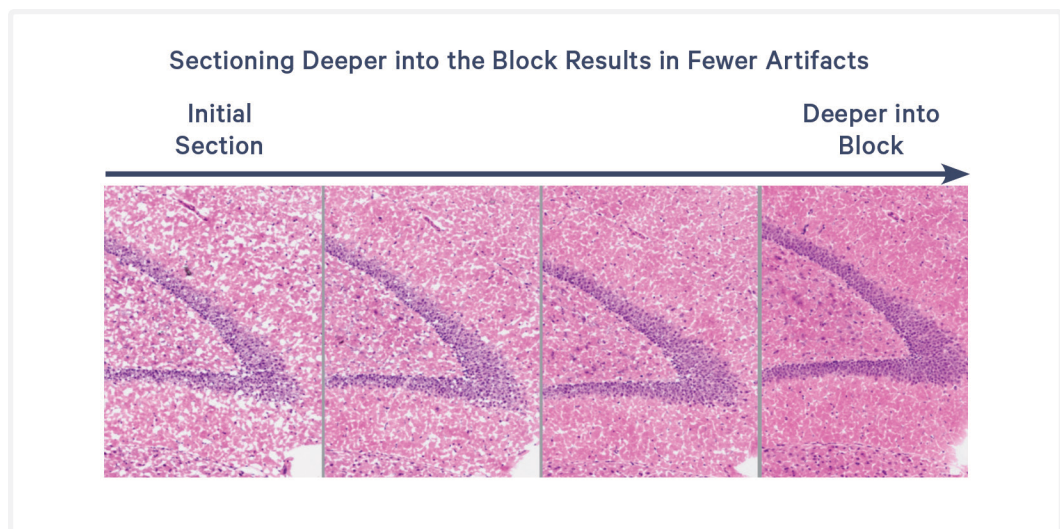
A slide ID may be given to a slide for later input into the Atera Instrument to assist with sample tracking. IDs must not exceed 33 characters and cannot contain !@#\$%&*)+=.

3.0 Get Started

Item	10x PN	Preparation & Handling	Storage	
Obtain				
☐	Tested Blank Slides	-	A list of tested slides can be found in the Atera Gene Expression Protocol Planner (CG001669). Slides should be chilled to cryostat temperature for ≥30 min . Warm slides will lead to quick melting of the sections.	Ambient
☐	Slide Mailer	-	Slide mailer should be chilled to cryostat temperature for ≥30 min .	Ambient
☐	OCT-embedded Tissue Block	-	OCT embedded tissue block stored at −80°C must be equilibrated to cryostat chamber temperature for 30 min before sectioning. If the tissue block is too cold, it will lead to section cracking. If the tissue block is too warm, it will lead to section compression or crumpling.	−80°C
☐	Specimen Chuck	-	Specimen chuck should be chilled to cryostat temperature for ≥5 min .	Ambient
☐	Cryostat	-	Turn cryostat on to chill chamber. Recommended sectioning temperature is −20°C for cryostat blade and −10°C for the specimen head. Follow manufacturer's manual for detailed operations.	Ambient
☐	Razor Blade <i>(optional, if scoring)</i>	-	Chill to cryostat temperature.	Ambient
☐	Anti-roll Plate <i>(optional)</i>	-	Anti-roll plate prevents rolling of tissue sections. Chill to cryostat temperature for ≥30 min . Warm anti-roll plate will damage tissue during sectioning. Optimize the position of anti-roll plate based on the tissue block size. If possible, adjust the position of anti-roll plate before reaching area of interest.	Ambient
☐	Hematoxylin	-	-	Ambient

3.1 Morphology Check

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



- Acquire a tissue section as described in [Tissue Sectioning on page 25](#) on a blank slide.

For this morphology check, fixation is not necessary. However, if fixation is desired, tissue can be fixed for 5–10 min in 10% NBF or 4% PFA followed by a wash in 1X PBS.
- Remove slide from the cryostat and immerse in a slide mailer filled with hematoxylin for **30 sec**.
- Immerse in a slide mailer filled with water for **20 sec**.
- Wipe the back of the slide with a laboratory wipe.
- Inspect tissue morphology under a microscope.
 - If morphology is ideal, cut and discard 3 sections with the section thickness set to 10 μm . Proceed to section placement.

Sections are discarded to move past the exposed block surface.

- If morphology is not ideal, repeat quality check with additional trimming of the block as needed.

3.2 Section Placement

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

Optional: Before section placement, trace the Sample Area onto the back of the slide with a marker. Using an unsupported marker may compromise assay performance. Consult the Atera Sample Placement Quick Reference Card (CG001675) for more information on the Sample Area.

- Once desired tissue section is obtained, carefully flatten it out by gently touching the surrounding OCT with cryostat brushes.
- Place the section within the Sample Area on the pre-equilibrated slide by gently touching the section with the active surface of the slide.



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

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



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

- Immediately place the slide with tissue section on the cryobar to freeze the section. Continue transferring sections onto the Sample Area.
- Transfer the slide containing tissue sections to a slide mailer pre-equilibrated in the cryostat.



- Store slides at **-80°C** for up to **4 weeks** or immediately proceed to the

Atera Gene Expression assay.



- Store up to two slides in a sealed container. Ensure slides are separated by a space to prevent damage. If using an unsealed slide mailer, store in a secondary sealed container, such as a resealable bag.
- Maintain slides containing sections in a cold and low moisture environment. DO NOT expose slides to room temperature as the resulting condensation will cause tissue disintegration.

Slide Shipping

- If needed, slides containing tissue sections can be shipped. See [Shipping Guidance on page 57](#) for detailed Shipping Guidelines.

Leftover Tissue Block Storage

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

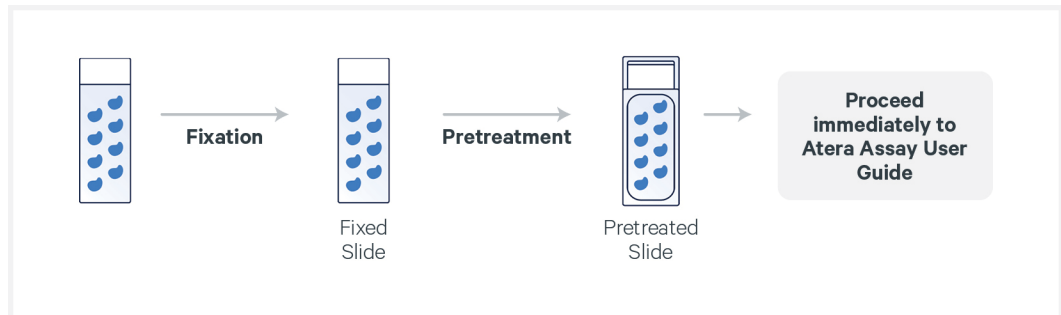
DO NOT let the block and tissue fully melt, as this will severely damage the tissue. Separation of the tissue block from the specimen stage is optional. The frozen tissue block can be stored attached to the specimen stage in a sealed container at -80°C .

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

4. Fixation & Pretreatment

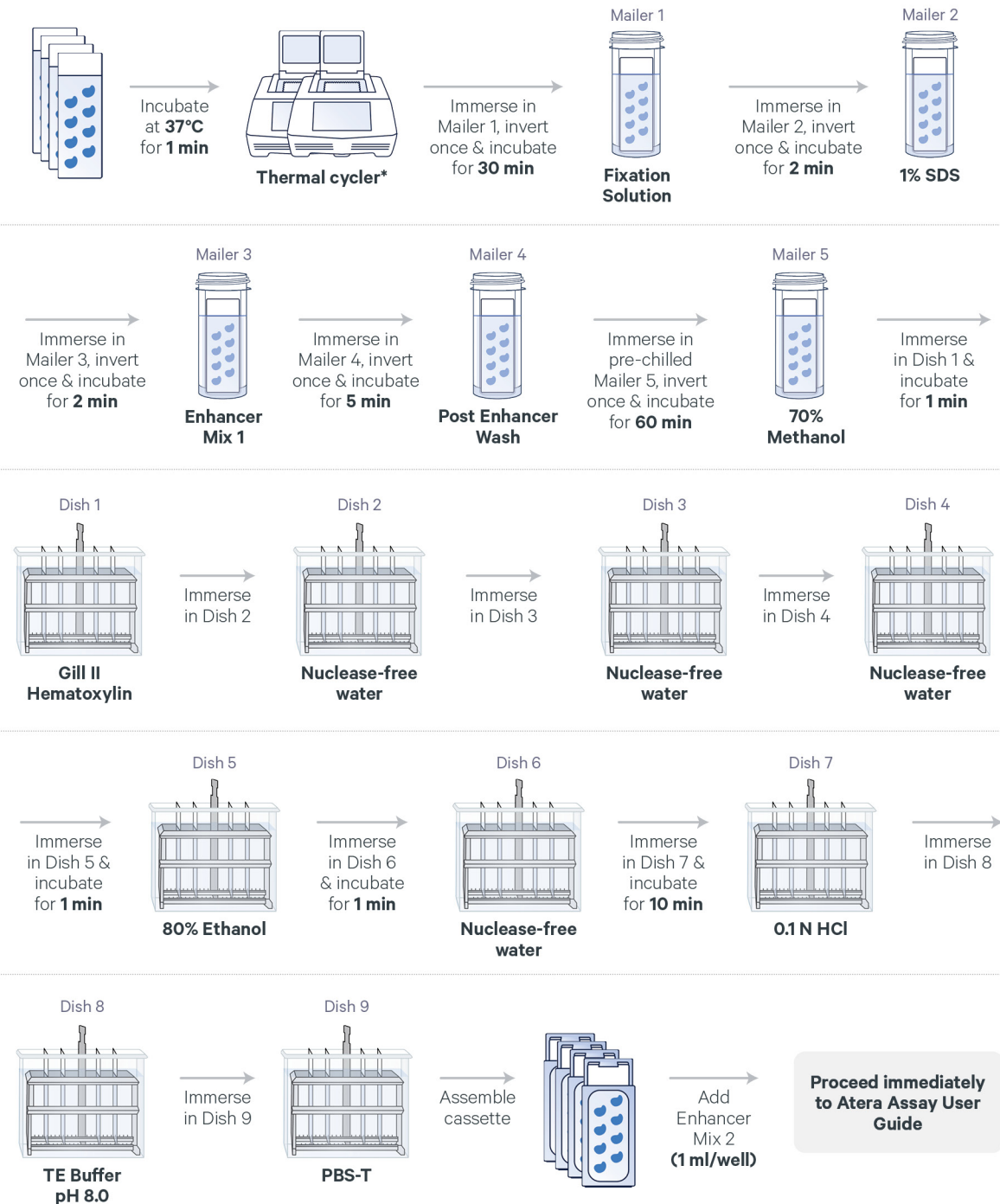
Overview

This chapter provides guidance on fixation and pretreatment of fresh frozen tissue sections on slides. These pretreatment steps have been optimized for downstream Atera chemistry and their purpose is proprietary.



Protocol Overview

Fixation & Pretreatment



*Keep the thermal cycler lid open during incubation

4.0 Get Started

Each 10x Genomics reagent tube is enough for two slides.

Fixation and Pretreatment Items	10x PN	Preparation & Handling	Storage
Maintain at Room Temperature			
 Additive A	220093	Thaw at room temperature, vortex, verify no precipitate, centrifuge briefly.	-20°C
		Additive A is required for the Atera Gene Expression User Guide (CG001672). DO NOT discard reagent after it is used in this Handbook.	
 Atera Fixation Additive	2001977	Thaw at 37°C for 5 min. Vortex, centrifuge briefly.	-20°C
Atera WTA, - Select, and Custom Panel Probes	-	DO NOT thaw until called for during Pretreatment. During Pretreatment, thaw appropriate probe panels at room temperature based on experimental needs. Pipette mix 10x and centrifuge briefly. DO NOT vortex. Consult the 10x Genomics Support Website for the most updated list of available panels and part numbers, as well as information on panel compatibility	-20°C
 Atera Hyb Buffer	2001879	DO NOT thaw until called for during Pretreatment. During Pretreatment, thaw at room temperature. Vortex and centrifuge briefly.	-20°C
See Prep and Handling			
 Atera Wash Enhancer L	2001978	Only retrieve Atera Wash Enhancer when indicated in the protocol. Preparation instructions are provided when needed.	-20°C

Fixation and Pretreatment Items	10x PN	Preparation & Handling	Storage
Obtain			
☐ Nuclease-free Water	-	-	Ambient
☐ 10X PBS	-	-	Ambient
☐ Paraformaldehyde	-	-	Ambient
☐ 10% SDS	-	-	Ambient
☐ 0.1 N HCl	-	-	Ambient
☐ Gill II Hematoxylin	-	-	Ambient
☐ Ethanol	-	-	Ambient
☐ Methanol	-	-	Ambient

Fixation and Pretreatment Items	10x PN	Preparation & Handling	Storage
☐ 10% Tween-20	-	-	Ambient
☐ TE Buffer, pH 8.0	-	-	Ambient
☐ Slide Mailers	-	If processing four slides, 10 mailers are required, with each mailer holding two slides. If processing two slides or less, 5 mailers are required. The volumes in this Handbook assume the use of the recommend part number as described in the Atera Gene Expression Protocol Planner (CG001669).	Ambient
☐ Slide Mailer Rack	-	-	Ambient
☐ Staining Dishes	-	Nine staining dishes are required, which can each hold up to 24 slides (if using the part number recommended in the Atera Gene Expression Protocol Planner, CG001669). If using a different staining dish, ensure that volumes are scaled appropriately and that the staining dish can process the desired number of slides simultaneously.	Ambient
☐ Slide Staining Rack	-	-	Ambient
☐ Forceps	-	-	Ambient
☐ Slides with fresh frozen tissue sections	-	Prepared according to earlier chapters in this Handbook.	-80°C
☐ Bucket with Dry Ice	-		
☐ Bucket with Ice	-		
☐ 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.	
☐ Atera Thermocycler Adaptor	-	See Tips & Best Practices	Ambient
☐ Atera Cassette	-	See Tips & Best Practices	Ambient

4.1 Preparation — Buffers

Prepare all buffers (except for Mailer 3 and Mailer 4) fresh before retrieving tissue sections from **-80°C**. Mailer 3 and 4 will be prepared during incubation in Mailer 1 (fixation). Some overage is included in all reagent preparations.

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

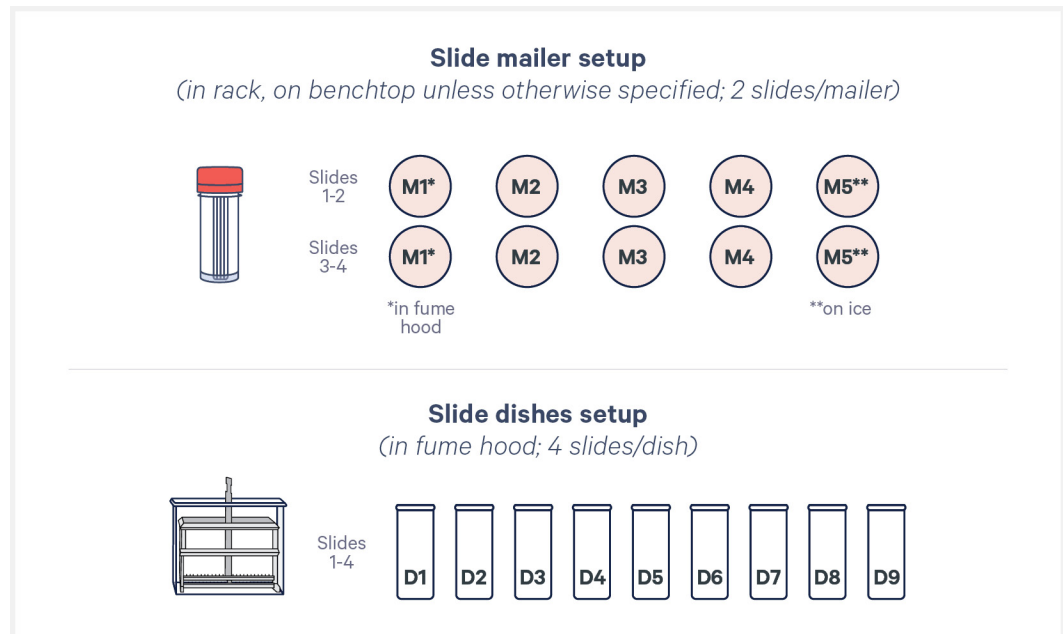
Ethanol preparations are only required if not using purchased ethanol that is already at the desired concentration.

The mailers recommended in the Atera Gene Expression Protocol Planner (CG001669) should be used for this protocol. However, the larger slide staining dishes may be replaced with smaller containers, provided that volumes are scaled appropriately and that they can process all slides simultaneously. If processing two slides or less, only one mailer is required for each step that calls for two mailers.

- a. Label ten total mailers and nine total staining dishes (if preparing four slides). Preparation instructions are in subsequent steps.

Items	Preparation & Handling
Fixation Solution	Label two slide mailers as Mailer 1.
1% SDS	Label two slide mailers as Mailer 2.
Enhancer Mix 1	Label two slide mailers as Mailer 3. These mailers will be filled during Mailer 1 incubation.
Post Enhancer Wash	Label two slide mailers as Mailer 4. These mailers will be filled during Mailer 1 incubation.
70% Methanol	Label two slide mailers as Mailer 5.
Gill II Hematoxylin	Label one slide dish as Dish 1.
Nuclease-free Water	Label four slide dishes as Dish 2, 3, 4, and 6
80% Ethanol	Label one slide dish as Dish 5.
0.1 N HCl	Label one slide dish as Dish 7.
TE Buffer, pH 8.0	Label one slide dish as Dish 8.
PBS-T	Label one slide dish as Dish 9.

Mailers will be arranged according to the following diagram.



- b. Prepare 1X PBS. Add reagents in the order listed. Invert gently to mix. Maintain at room temperature. This volume of 1X PBS is sufficient for washes in all subsequent steps of this Handbook, as well as the Atera Gene Expression User Guide (CG001672).

1X PBS					
Items	Stock	Final	1X (ml)	2X (ml)	4X (ml)
Nuclease-free water	-	-	292.9	310.9	373.7
RNase-free PBS, pH 7.4	10X	1X	32.6	34.5	41.5
Total	-	-	325.5	345.4	415.2

- c. Using 1X PBS from the previous step, prepare PBS-Tween (PBS-T). Add reagents in the order listed. Invert gently to mix. Maintain at room temperature. This volume of PBS-T is sufficient for washes in all subsequent steps of this Handbook, as well as the Atera Gene Expression User Guide (CG001672).

PBS-T					
Items	Stock	Final	1X (ml)	2X (ml)	4X (ml)
1X PBS	-	-	292.5	312.4	350.2
Tween-20	10%	0.05%	1.5	1.6	1.8
Total	-	-	294.0	314.0	352.0



Pipette Tween-20 slowly to fully dispense from pipette tip and to avoid formation of air bubbles.

- d. Using 1X PBS from step 4.1b, prepare Fixation Solution according to the appropriate table below. Add reagents in the order listed. Invert gently to mix. Add 10 ml to each Mailer 1 and close the cap.

Fixation Solution for Mailer 1					
Items	Stock	Final	1 Mailer (ml)	2 Mailers (ml)	
1X PBS	-	-	7.2	14.4	
Paraformaldehyde	16%	4%	2.6	5.3	
 Atera Fixation Additive	-	-	0.7	1.3	
Total	-	-	10.5	21.0	

- e. Prepare 1% SDS according to the table below. Vortex 10% SDS stock thoroughly. Verify no precipitate in the 10% SDS stock — if necessary, heat stock to 37°C to remove precipitate. Add reagents in the order listed. Invert gently to mix. Add 10 ml to each Mailer 2 and close the cap.

1% SDS for Mailer 2					
Items	Stock	Final	1 Mailer (ml)	2 Mailers (ml)	
Nuclease-free water	-	-	9.5	18.9	
SDS	10%	1%	1.1	2.1	
Total	-	-	10.6	21.0	

- f. Prepare 70% methanol. Add reagents in the order listed. Invert gently to mix. Add 10 ml to each Mailer 5 and close the cap. Chill Mailer 5 on ice for **30 min** before beginning fixation.

70% Methanol for Mailer 5				
Items	Stock	Final	1 Mailer (ml)	2 Mailers (ml)
Methanol	100%	70%	7.4	14.7
Nuclease-free water	-	-	3.2	6.3
Total	-	-	10.6	21.0

- g. Dispense **250 ml** Gill II Hematoxylin into Dish 1 and apply lid.
- h. Dispense **250 ml** nuclease-free water into Dish 2, 3, and 4. Apply lids.
- i. Prepare 80% ethanol. Add reagents in the order listed. Invert gently to mix. Dispense 250 ml into Dish 5 and apply lid.

80% Ethanol for Dish 5			
Items	Stock	Final	1 Slide Staining Dish (ml)
Ethanol	100%	80%	200
Nuclease-free water	-	-	50
Total	-	-	250

- j. Dispense **250 ml** nuclease-free water into Dish 6 and apply lid.
- k. Dispense **250 ml** 0.1 N HCl into Dish 7 and apply lid.
- l. Dispense **250 ml** TE Buffer, pH 8.0 into Dish 8 and apply lid.
- m. Dispense **250 ml** PBS-T into Dish 9 and apply lid.
- n. Arrange mailers and dishes according to the diagram at the beginning of this step.

4.2 Slide Preparation

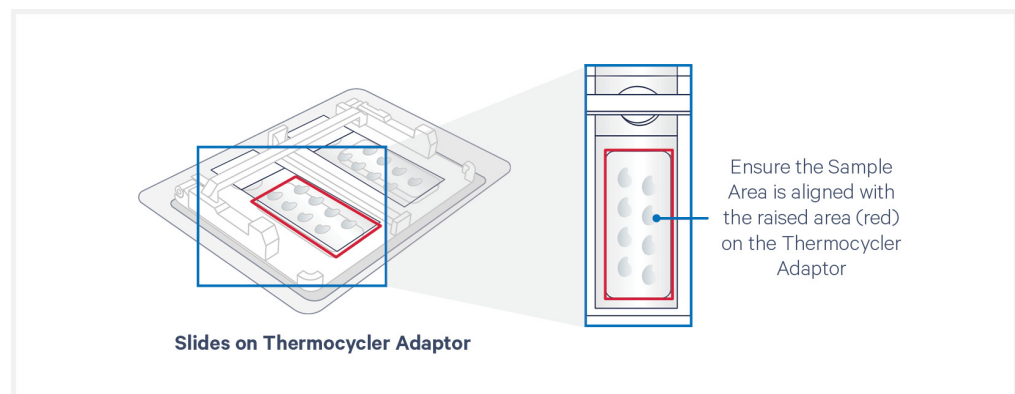
- a. Place Atera Gene Expression Thermocycler Adaptors in thermal cyclers set to incubate at **37°C**. DO NOT close the lid.

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

- b. Prepare an ice bucket of dry ice.
- c. Remove slide mailer containing stored fresh frozen tissue 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 a pair of forceps and immediately place in pre-chilled empty slide mailer on dry ice.*

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



4.3 Fixation

- a. **Immediately** remove slides from thermal cyclers following incubation. Gently immerse slides in Mailer 1 using slide forceps, two slides per container. Close mailer. Invert mailer once.



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

See [Tips & Best Practices](#) for guidance on properly immersing slides into mailers.



Ensure Mailer 5s (70% Methanol) are pre-chilled on ice before proceeding to next step. Complete remaining steps in this section during fixation incubation.

- b. Incubate slides in Mailer 1 for **30 min** at **room temperature**.
- c. During Mailer 1 incubation, incubate Atera Wash Enhancer L at **65°C** for **10 min** or until solution is reconstituted to a dark green solution with no visible particulates. If using a temperature-controlled instrument with a 1.5 ml capacity, additional thawing time or mixing may be required.

DO NOT exceed 20 min at **65°C**. Store at room temperature until use. Gently invert before use.

Atera Wash Enhancer L color change over time is normal. Thaw one tube for every two slides.

Atera Wash Enhancer Before and After Incubation



- d. Prepare Enhancer Mix 1 according to the table below, adding reagents in the order listed (some overage is included). Vortex. Add 10 ml to each Mailer 3. Maintain at room temperature.



Atera Wash Enhancer L is also used later in the workflow. Maintain at room temperature.

Enhancer Mix 1 color change over time is normal.

Enhancer Mix 1 for Mailer 3				
	Stock	Final	1 Mailer (ml)	2 Mailers (ml)
1X PBS	-	-	9.5	18.9
 Atera Wash Enhancer L	-	-	1.1	2.1
Total	-	-	10.6	21.0

- e. Prepare Post Enhancer Wash according to the table below, adding reagents in the order listed (some overage is included). Vortex. Add 10 ml to each Mailer 4. Maintain at room temperature.



*Additive A is also used in the Atera Gene Expression User Guide. **DO NOT** discard reagent after using during the sample preparation workflow.*

Post Enhancer Wash for Mailer 4				
	Stock	Final	1 Mailer (ml)	2 Mailers (ml)
1X PBS	-	-	10.3	20.6
 Additive A	-	-	0.2	0.4
Total	-	-	10.5	21.0

4.4 Pretreatment

- a. Gently immerse slides in Mailer 2 (1% SDS) using slide forceps. Close mailer tightly. Invert mailer once. Incubate for **2 min** at **room temperature**.
- b. Gently immerse slides in Mailer 3 (Enhancer Mix 1) using slide forceps. Close mailer tightly. Invert mailer once. Incubate for **2 min** at **room temperature**.
- c. Gently immerse slides in Mailer 4 (Post Enhancer Wash) using slide forceps. Close mailer tightly. Invert mailer once. Incubate for **5 min** at **room temperature**.
- d. Gently immerse slides in chilled Mailer 5 (70% Methanol) using slide forceps. Close mailer tightly. Invert mailer once. Incubate for **60 min** on **ice**.



Begin thawing Atera Hyb Buffer and relevant probes during pretreatment. These are needed for Probe Hybridization in Atera Gene Expression User Guide (CG001672). After the completion of Pretreatment, the Probe Hybridization step in the User Guide must be started immediately.

If needed, move Mailer 5 closer to Dish 1.

- e. Transfer slides to slide staining rack.
- f. Gently immerse slides in Dish 1 (Gill II Hematoxylin). Incubate for **1 min** at **room temperature**.
- g. Gently immerse slides 1x in Dish 2 (nuclease-free water).
- h. Gently immerse slides 1x in Dish 3 (nuclease-free water).
- i. Gently immerse slides 1x in Dish 4 (nuclease-free water).
- j. Remove lid from Dish 5, then gently immerse slides in Dish 5 (80% Ethanol). Incubate for **1 min** at **room temperature**.
- k. Gently immerse slides in Dish 6 (nuclease-free water). Incubate for **1 min** at **room temperature**.
- l. Gently immerse slides in Dish 7 (0.1 N HCl). Incubate for **10 min** at **room temperature**.
- m. Prepare Enhancer Mix 2 according to the table below during Dish 7 incubation, adding reagents in the order listed (some overage is included). Pipette mix Atera Wash Enhancer L before adding to tube. Vortex. Maintain at room temperature.

Enhancer Mix 2 color change over time is normal.

Enhancer Mix 2		Stock	Final	1X (ml)	2X (ml)	4X (ml)
	1X PBS	-	-	1.00	2.00	4.00
	Atera Wash Enhancer L	-	-	0.05	0.10	0.20
	Total	-	-	1.05	2.1	4.2

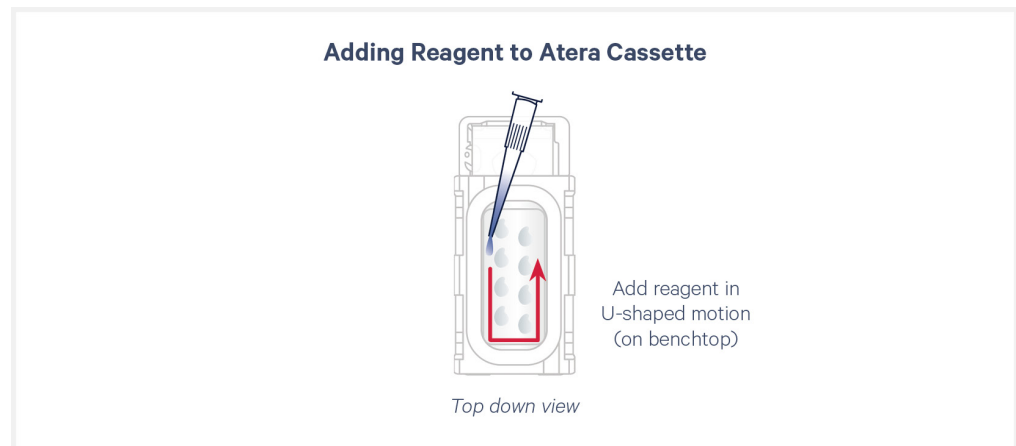
- n. Gently immerse slides 1x in Dish 8 (TE Buffer, pH 8.0).
- o. Gently immerse slides 1x in Dish 9 (PBS-T).
After immersion, remove any marker annotations using a lint-free laboratory wipe and 70% ethanol or isopropanol.
- p. Place one slide in a cassette and add **1 ml** Enhancer Mix 2 to the well.
Perform one slide at a time to minimize risk of slide drying.
- q. Repeat for remaining slides.



DO NOT open Atera Cassette Inserts.



Consult the Atera Cassette Assembly, Disassembly, and Use Quick Reference Card (CG001674) for guidance on Atera Cassette Assembly. Work quickly to avoid drying out of tissue sections.



- r. *Photograph the slide in the cassette without the gasket from above against a black background. This image can be used for comparison purposes to help identify potential issues encountered during the workflow. Work quickly as this is not a safe stopping point.*



Observe the Atera Cassette for any leaks. If leaks occur, slide may need to be reassembled into the cassette. Consult the Atera Cassette Assembly, Disassembly, and Use Quick Reference Card (CG001674) for cassette assembly and removal instructions.

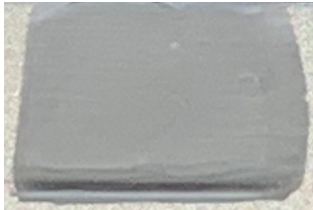


- s. Place cassettes on Thermocycler Adaptors.
- t. Proceed to the Atera Gene Expression User Guide (CG001672).
This is not a safe stopping point.

Troubleshooting

Impact of Cryostat Specimen Head Temperatures on Tissue Tearing

-10°C



Normal Section

-14°C



-20°C

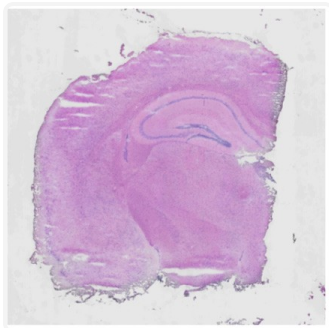


-30°C

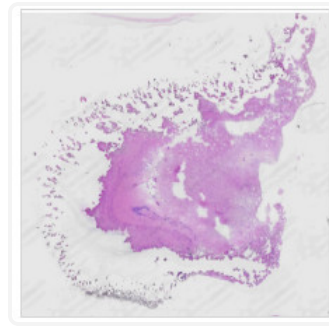


Tissue section has significant tearing.

Impact of Condensation on Tissue Sections



No Condensation. Intact tissue section.

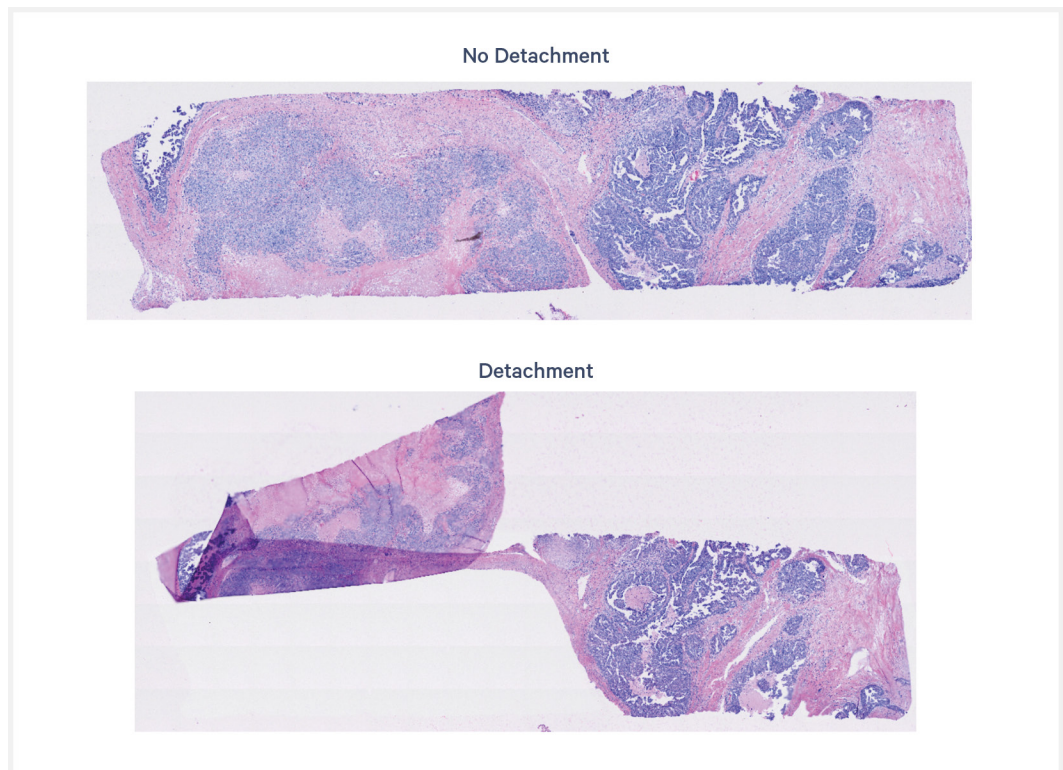


Tissue degraded due to condensation. DO NOT leave slides at room temperature, especially with fresh sections as the resulting condensation will cause tissue disintegration.

Tissue Detachment

Tissue detachment may result in a lack of decodable data in the region where detachment occurred. If the tissue has folded on itself, this may also cause elevated signal in the overlapping areas. To keep track of possible detachment, photograph the slides after pretreatment.

If detachment is observed during the workflow (>30% tissue detachment), 10x Genomics does not recommend proceeding with the workflow. To mitigate detachment, place difficult tissues (low quality samples, TMAs, etc.) on Schott Nexterion Slide H - 3D Hydrogel Coated Slides (Schott North America, PN-1800434) or Tissue Adhesion Slides (10x Genomics, PN-1001228).



Appendix

Fixation and Quality Assessment

Tissue sections should be fixed before quality assessment (DAPI and H&E staining)

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

DAPI Staining Items		10x PN	Preparation & Handling	Storage
Obtain				
☐	DAPI Solution	-	Use any preferred DAPI stock solution. Dilute DAPI stock solution to 5 µg/ml in PBS-T to generate DAPI solution. 1.5 ml of DAPI solution is enough for two slides.	Ambient
☐	10X PBS	-	Prepare 1X PBS using nuclease-free water.	Ambient
☐	Slide Mailer	-	-	Ambient
☐	Glycerol Mounting Medium	-	The dilution below is not necessary if stock glycerol is already at 85%. Invert to mix. Briefly centrifuge to remove bubbles.	

Glycerol Mounting Medium <i>1 slide = 1 Tissue Slide</i>	Stock	Final	1 Slide (µl)	2 Slides +15% (µl)	4 Slides +15% (µl)
Glycerol	100%	85%	127.5	293.3	586.5
Nuclease-free Water	-	-	22.5	51.7	103.5
Total	-	-	150.0	345.0	690.0

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

Preparation — Buffers

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

TIPS

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

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

1X PBS			
Items	Stock	Final	Total Amount (ml)
Nuclease-free water	-	-	18
RNase-free PBS, pH 7.4	10X	1X	2
Total	-	-	20

- b. Using 1X PBS from step a, prepare Fixation Solution. Add reagents in the order listed. Invert gently to mix. Maintain at room temperature.

Fixation Solution			
Items	Stock	Final	Total Amount (ml)
1X PBS	-	-	15
Paraformaldehyde	16%	4%	5
Total	-	-	20

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

Slide Preparation

- a. Place Atera Gene Expression Thermocycler Adaptors in thermal cyclers set to incubate at **37°C**. DO NOT close the lid.

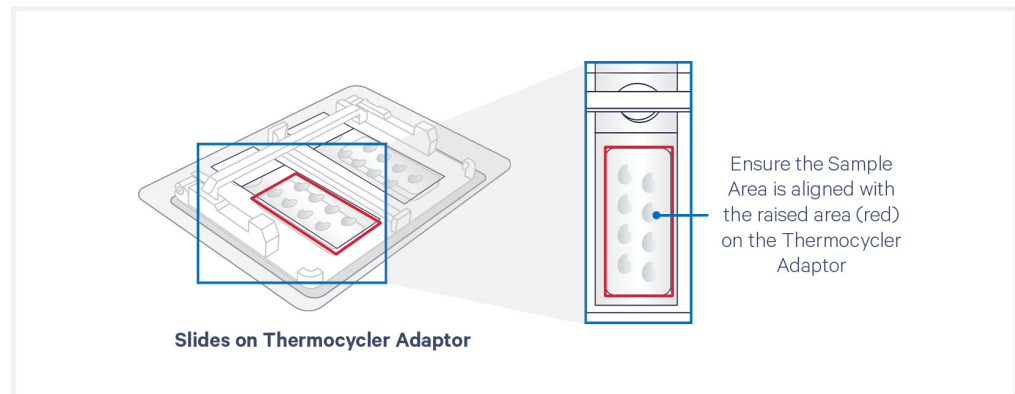
Ready the Fixation Solution Mailer and a timer set to 1 min, which are needed in the following steps.

- b. Prepare an ice bucket of dry ice.

- c. Remove slide mailer containing stored fresh frozen tissue 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 a pair of forceps and immediately place in pre-chilled empty slide mailer on dry ice.*

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



Fixation

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



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

DAPI Staining

DAPI staining is optional. If only performing H&E staining, skip to [H&E Preparation](#).

- a. Place tissue slide on a flat, clean, nonabsorbent work surface.
- b. Add **750 µl** DAPI solution per slide to uniformly cover all tissue sections.

- c. Incubate **1 min** in the dark at **room temperature**.
- d. Discard reagent by holding slide at an angle with the bottom edge in contact with a laboratory wipe.
- e. Add **1 ml** 1X PBS to uniformly cover all tissue sections.
- f. Incubate **1 min** in the dark at **room temperature**.
- g. Discard reagent by holding slide at an angle with the bottom edge in contact with a laboratory wipe.
- h. Repeat steps e-g twice more for a total of three washes.
- i. Wipe excess liquid from the front and back of the slide without touching the tissue section.

Coverslipping

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

- a. Place slide on a flat, clean, non-absorbent work surface.
- b. Using a **wide-bore** pipette tip, add **150–200 µl** Glycerol Mounting Medium to uniformly cover all tissue sections on the slide.
- c. Apply the coverslip at an angle on one end of the slide. Slowly lower the coverslip, without introducing bubbles. Allow mounting media to spread and settle.
- d. If needed, remove any large excess of mounting media by carefully wicking away from the edge of the coverslip with a lint-free laboratory wipe. Be careful not to move coverslip and disturb the tissue.
- e. Once coverslipping is complete, **immediately** proceed with imaging. See [DAPI Quality Assessment](#) for information on evaluating DAPI staining. Underexposure may result in inaccurate assessment of DAPI quality. Begin by overexposing samples, then reducing exposure until the resolution improves. If possible, process a control section in parallel from a block that has yielded good data.

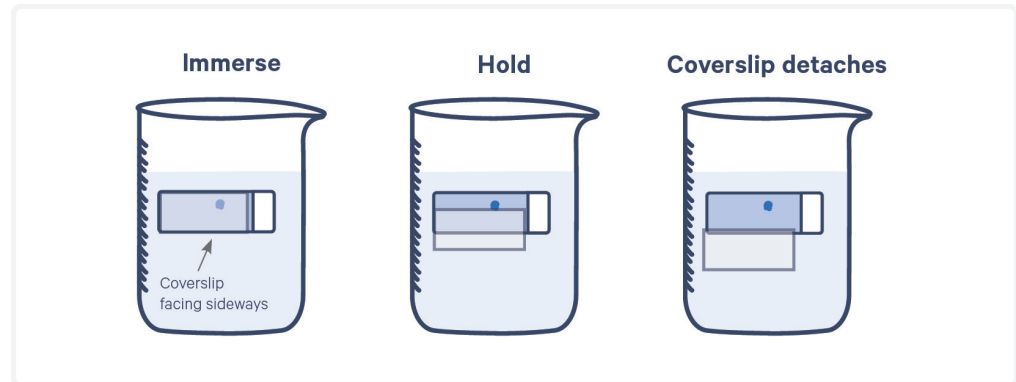
Coverslip Removal

- a. Dispense **800 ml** Milli-Q water in a beaker. Up to 10 slides may be processed using this beaker.

- b. Immerse slides sideways in the beaker containing **800 ml** water with the coverslipped surface fully sideways to prevent the coverslip from dragging across the tissue.
- c. Hold slides in water until the coverslip slowly separates away from the slide.



To avoid tissue section damage or detachment, DO NOT move the slide up and down, shake forcibly, or manually move the coverslip.



- d. Gently immerse slides 30x in water to ensure all Mounting Medium is removed.
- e. Wipe the back of the slide with a laboratory wipe. Place on a flat, clean, nonabsorbent work surface and air dry for **5 min**.
- f. Proceed with H&E Staining.

H&E Preparation

TIPS

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

- a. Filter Hematoxylin & Eosin solutions using filter paper before starting H&E Staining protocol.
- b. Prepare seventeen total coplin jars for H&E Staining steps.

For H&E Staining	
Items	Preparation & Handling
Hematoxylin Solution	Label one coplin jar as Hematoxylin Jar. Fill to capacity with Gill II Hematoxylin Solution.
Bluing Solution	Label one coplin jar as Bluing Solution Jar. Fill to capacity with Bluing Solution.
70% Ethanol	Label one coplin jar as 70% Ethanol Jar. Fill to capacity with 70% ethanol.
95% Ethanol	Label three coplin jars as 95% Ethanol Jar 1, 2, and 3. Fill to capacity with 95% ethanol.
Eosin Solution	Label one coplin jar as Eosin Solution Jar. Fill to capacity with Eosin Solution.
100% Ethanol	Label two coplin jars as 100% Ethanol Jar 1 and 2. Fill to capacity with 100% Ethanol.
Xylene	Label two coplin jars as Xylene Jar 1 and 2. Fill to capacity with Xylene.
Milli-Q Water	Label six coplin jars as Milli-Q Water Jar 1, 2, 3, 4, 5, and 6. Fill to capacity with Milli-Q Water.

TIPS

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

H&E Staining

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

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

TIPS

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

- b. Gently immerse slide in the Hematoxylin Solution Jar for **2 min** at **room temperature**.



Substitution of Hematoxylin with a brand other than the recommended option may lead to tissue staining that is darker than anticipated following 2 min incubation.

- c. Gently immerse slide 10x in the Milli-Q Water Jar 2.
- d. Gently immerse slide 10x in the Milli-Q Water Jar 3.
- e. Gently immerse slide 10x in the Milli-Q Water Jar 4.
- f. Gently immerse slide in the Bluing Solution Jar for **1 min** at **room temperature**.
- g. Gently immerse slide 10x in the Milli-Q Water Jar 5.
- h. Gently immerse slide 10x in the Milli-Q Water Jar 6.
- i. Gently immerse slide in the 70% Ethanol Jar for **1 min** at **room temperature**.
- j. Gently immerse slide in the 95% Ethanol Jar 1 for **1 min** at **room temperature**.
- k. Gently immerse slide in the Eosin Solution Jar for **2 min** at **room temperature**.
- l. Gently immerse slide in the 95% Ethanol Jar 2 for **30 sec** at **room temperature**.
- m. Gently immerse slide in the 95% Ethanol Jar 3 for **30 sec** at **room temperature**.
- n. Gently immerse slide in the 100% Ethanol Jar 1 for **30 sec** at **room temperature**.
- o. Gently immerse slide in the 100% Ethanol Jar 2 for **30 sec** at **room temperature**.
- p. Gently immerse slide in the Xylene Jar 1 for **3 min** at **room temperature**.
- q. Gently immerse slide in the Xylene Jar 2 for **3 min** at **room temperature**.

Coverslipping

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



The mounting medium used for mounting H&E-stained sections is different than what was used to mount slides after DAPI staining. Ensure that the appropriate mounting media is used.

- a. Place slide on a flat, clean, non-absorbent work surface.
 - b. Using a **wide-bore** pipette tip, add **150–200 µl** mounting media to uniformly cover all tissue sections on the slide.
 - c. Apply the coverslip at an angle on one end of the slide. Slowly lower the coverslip, without introducing bubbles. Allow mounting media to spread and settle.
 - d. If needed, remove any large excess of mounting media by carefully wicking away from the edge of the coverslip with a lint-free laboratory wipe. Be careful not to move coverslip and disturb the tissue.
 - e. Dry the coverslipped slide for **30 min** at **room temperature**.
-
- f. Once coverslipping is complete, **immediately** proceed with imaging. Samples may be stored temporarily at **4°C** and imaged within 3 days after coverslipping. For long-term storage after imaging, maintain at **room temperature in the dark**.

Shipping Guidance

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

Document Revision Summary

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Revision Date	Sept 2026
Description of Changes	Initial Release

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