Atera Gene Expression - Post-Atera H&E Staining

Introduction

The Atera Gene Expression workflow is designed to measure mRNA in tissue sections derived from formalin fixed & paraffin embedded (FFPE) and fresh frozen (FF) tissue samples. Following an Atera Instrument run, tissue sections may be optionally processed for Hematoxylin & Eosin (H&E) staining. This document provides guidance on H&E staining for both FFPE and FF tissue sections.

Additional Guidance

Consult the 10x Genomics Support website for additional resources.

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 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

Pipette Calibration

Follow manufacturer's calibration and maintenance schedules.

Slide Storage

- Store analyzed Atera Cassettes in PBS-T at 4°C following an Atera Instrument run. Consult the Atera Instrument User Guide (CG001673) for more information about post-Atera storage conditions.
- Proceed to H&E Staining within 3 days following an instrument run.
- For long-term storage after H&E staining and imaging, maintain at room temperature in the dark.

Atera Cassette Handling

- Consult the Atera Cassette Handling Quick Reference Card (CG001674) for information on cassette disassembly. Other topics (not relevant to this Demonstrated Protocol) covered include:
  - Atera Cassette Lid application and removal
  - Atera Cassette Insert application and removal
  - Thermocycler Adaptor handling

Slide Incubation

- Place slides with label toward the top of the staining dish or coplin jar 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 staining dish or coplin jar. Slides with tissues in the first or last position may get scratched.
- Buffer volume in slide dishes and coplin jars does not change if processing more than one slide.

H&E Staining

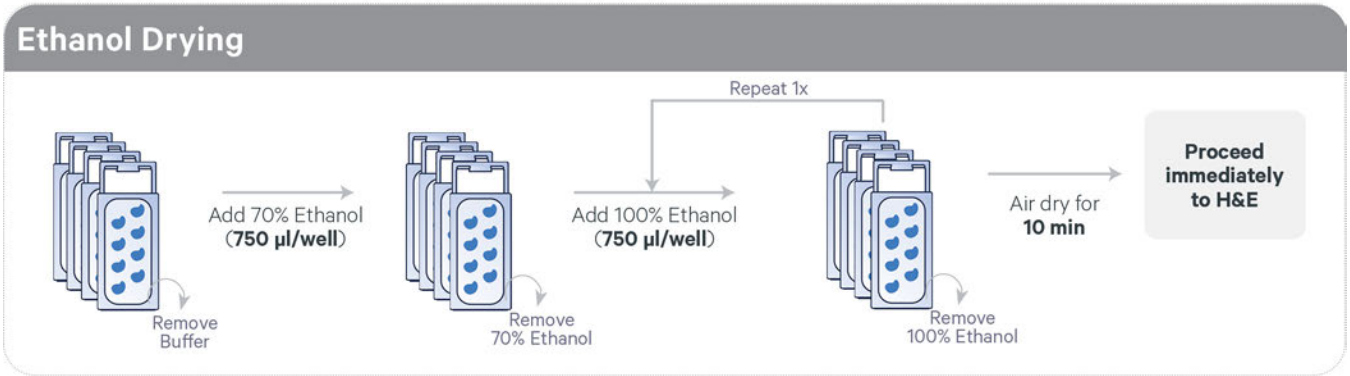
- Test out H&E staining protocol on a serial section placed on a blank slide before staining slides processed with Atera.
- Any H&E staining protocol may be used, but results may vary from the protocol described in this document.
- If staining is too light or dark, check reagent concentration and consider optimizing staining time of Hematoxylin and Eosin solutions.
- Use fresh staining reagents, as the pH of reagents (e.g., Hematoxylin) can change over time and lead to suboptimal staining.
- Filter Hematoxylin and Eosin solutions using filter paper before starting H&E staining.
- Replace ethanol frequently as any dye leftover in ethanol can stain tissues.

1. H&E Staining

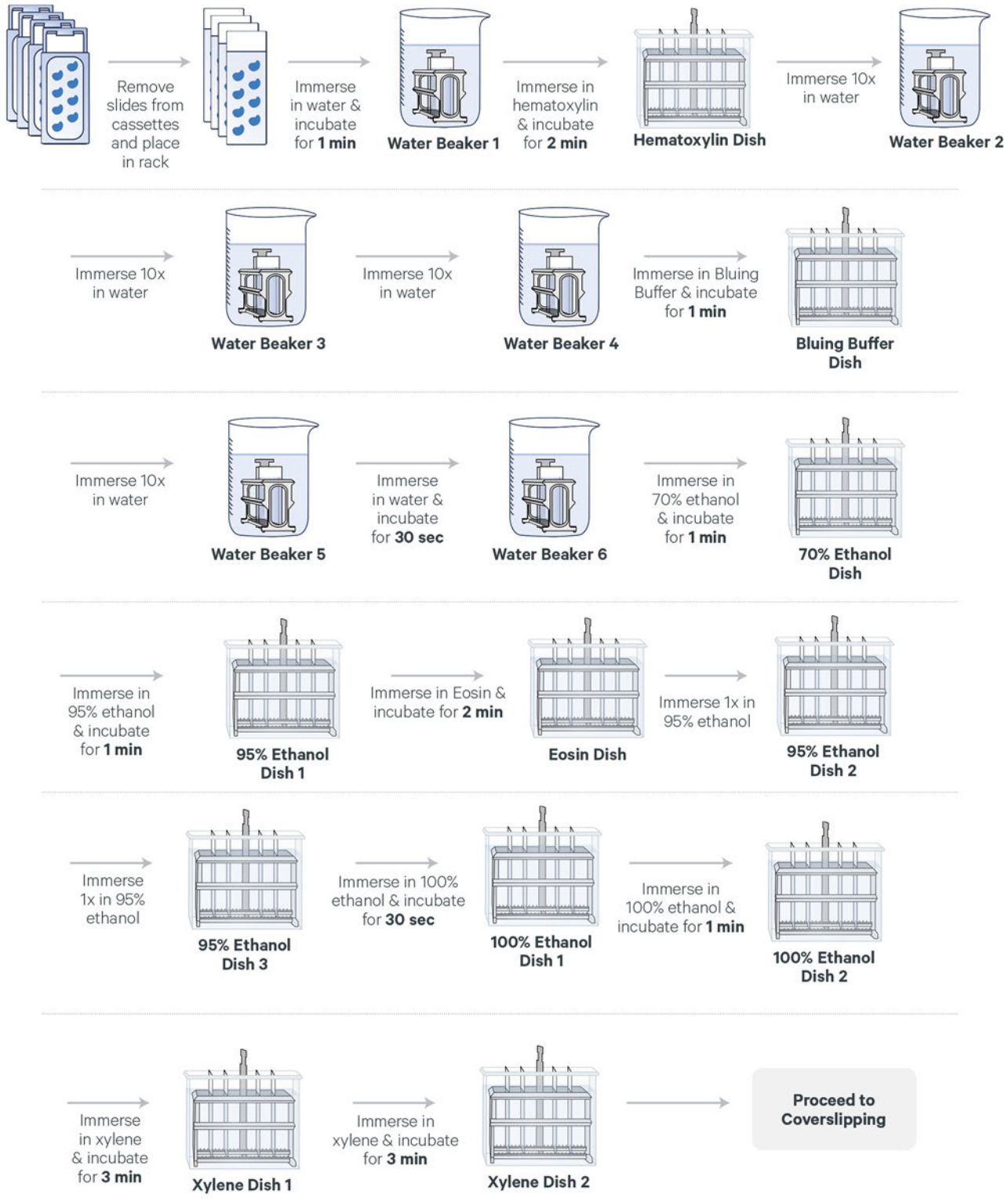
Overview

This chapter provides guidance on H&E staining of slides containing FFPE or fresh frozen tissue sections. Tissue sections are first dried, then stained with Hematoxylin and Eosin staining solutions. Next, tissues are dried further and cleared using a combination of ethanol and xylene washes. Finally, samples are coverslipped to prepare for imaging. Proceed to imaging following the Post-Atera H&E Staining workflow.

Protocol Overview



H&E Staining


Remove slides from cassettes and place in rack
Immerse in water & incubate for 1 min
Water Beaker 1
Immerse in hematoxylin & incubate for 2 min
Hematoxylin Dish
Immerse 10x in water
Water Beaker 2
Immerse 10x in water
Water Beaker 3
Immerse 10x in water
Water Beaker 4
Immerse in Bluing Buffer & incubate for 1 min
Bluing Buffer Dish
Immerse 10x in water
Water Beaker 5
Immerse in water & incubate for 30 sec
Water Beaker 6
Immerse in 70% ethanol & incubate for 1 min
70% Ethanol Dish
Immerse in 95% ethanol & incubate for 1 min
95% Ethanol Dish 1
Immerse in Eosin & incubate for 2 min
Eosin Dish
Immerse 1x in 95% ethanol
95% Ethanol Dish 2
Immerse 1x in 95% ethanol
95% Ethanol Dish 3
Immerse in 100% ethanol & incubate for 30 sec
100% Ethanol Dish 1
Immerse in 100% ethanol & incubate for 1 min
100% Ethanol Dish 2
Immerse in xylene & incubate for 3 min
Xylene Dish 1
Immerse in xylene & incubate for 3 min
Xylene Dish 2
Proceed to Coverslipping

Get Started

H&E Staining Items		Preparation & Handling	Storage
Obtain			
☐	Gill II Hematoxylin	-	Ambient
☐	Eosin Y	-	Ambient
☐	Bluing Buffer	-	Ambient
☐	Mounting Media	-	Ambient
☐	Coverslips	Optional, if desired for slide handling.	Ambient
☐	Xylene	-	Ambient
☐	100% Ethanol	-	Ambient
☐	Milli-Q Water	-	Ambient
☐	Forceps	Optional, if desired for slide handling.	Ambient
☐	Coplin jars/Staining dishes	-	Ambient
☐	Slide Staining Rack	Only if using staining dishes. Slide rack should fit into the chosen slide staining dishes.	Ambient
☐	1 L Beakers	Six 1 L beakers are required.	Ambient

1.0 Preparation – Buffers

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

a. Label 11 total staining dishes and six beakers. Coplin jars may also be used instead of staining dishes. If using coplin jars, scale volumes accordingly.

Items		Preparation & Handling
☐	Milli-Q Water	Label six 1 L beakers as Water beaker 1, 2, 3, 4, 5, and 6.
☐	70% Ethanol	Label one staining dish as 70% Ethanol Dish.
☐	95% Ethanol	Label three staining dishes as 95% Ethanol Dish 1, 2, and 3.
☐	100% Ethanol	Label two staining dishes as 100% Ethanol Dish 1 and 2.
☐	Xylene	Label two staining dishes as Xylene Dish 1 and 2.
☐	Gill II Hematoxylin	Label one staining dish as Hematoxylin Dish.
☐	Eosin Y	Label one staining dish as Eosin Dish.
☐	Bluing Buffer	Label one staining dish as Bluing Buffer Dish.

b. Dispense **800 ml** Milli-Q Water into Water Beakers 1–6.

c. Prepare 95% Ethanol. Add reagents in the order listed. Invert gently to mix. Fill each 95% ethanol staining dish with 250 ml 95% ethanol.

95% Ethanol				
Items		**Stock**	**Final**	**3 Staining Dishes (ml)**
	Ethanol	100%	95%	712.5
	Milli-Q Water	-	-	37.5
	Total	**-**	**-**	**750.0**

d. Prepare 70% Ethanol. Add reagents in the order listed. Invert gently to mix. Fill 70% ethanol staining dish with 250 ml 70% ethanol.

70% Ethanol				
Items		**Stock**	**Final**	**1 Staining Dish (ml)**
	Ethanol	100%	70%	175
	Milli-Q Water	-	-	75
	Total	**-**	**-**	**250**

e. Dispense **250 ml** 100% ethanol into 100% Ethanol Dish 1 and 2.

f. Dispense **250 ml** xylene into Xylene Dish 1 and 2.

g. Dispense **250 ml** Gill II Hematoxylin into Hematoxylin Dish.

h. Dispense **250 ml** Eosin into Eosin Dish.

i. Dispense **250 ml** Bluing Buffer into Bluing Buffer Dish.

1.1 Ethanol Drying

a. Prepare 70% Ethanol shortly before use. Add reagents in the order listed. Pipette mix 10x and centrifuge briefly.

70% Ethanol	Stock	Final	1X +10% (µl)	2X +10% (µl)	4X +10% (µl)
Milli-Q Water	-	-	247	495	990
Ethanol	100%	70%	578	1,155	2,310
Total		**-**	**825**	**1,650**	**3,300**

b. Retrieve Atera Cassettes.

c. Remove all buffer from each well.

d. Add **750 µl** 70% Ethanol to each well. Incubate for **1 min** at **room temperature**. Remove all 70% Ethanol.

Two 100% Ethanol washes:

e. **Wash 1:** Add **750 µl** 100% Ethanol to each well. Incubate for **1 min** at **room temperature**. Remove all 100% Ethanol.

f. **Wash 2:** Add **750 µl** 100% Ethanol to each well. Incubate for **1 min** at **room temperature**. Remove all 100% Ethanol.

g. Air dry slides in cassettes for **10 min** at **room temperature**.

h. **Immediately** proceed to next step.

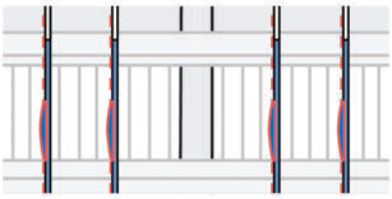
1.2 H&E Staining

a. Remove slides from Atera Cassettes.

TIPS

Consult the Atera Cassette Handling Quick Reference Card (CG001674) for guidance on Atera Cassette disassembly.

b. Add slides to slide staining rack. Leave space between each slide.



c. Gently immerse slides in Water Beaker 1 and incubate for **1 min**.

d. Gently immerse slides in Hematoxylin Dish and incubate for **2 min**.

e. Immerse slides 10x in Water Beaker 2.

f. Immerse slides 10x in Water Beaker 3.

g. Immerse slides 10x in Water Beaker 4.

h. Gently immerse slides in Bluing Buffer Dish and incubate for **1 min**.

i. Immerse slides 10x in Water Beaker 5.

j. Gently immerse slides in Water Beaker 6 and incubate for **30 sec**.

k. Gently immerse slides in 70% Ethanol Dish and incubate for **1 min**.

l. Gently immerse slides in 95% Ethanol Dish 1 and incubate for **1 min**.

m. Gently immerse slides in Eosin Dish and incubate for **2 min**.

n. Immerse slides 1x in 95% Ethanol Dish 2.

o. Immerse slides 1x in 95% Ethanol Dish 3.

p. Gently immerse slides in 100% Ethanol Dish 1 and incubate for **30 sec**.

q. Gently immerse slides in 100% Ethanol Dish 2 and incubate for **1 min**.

r. Gently immerse slides in Xylene Dish 1 and incubate for **3 min**.

When immersing slides in xylene, ensure that the tissue sections are completely submerged. DO NOT submerge the label.

s. Gently immerse slides in Xylene Dish 2 and incubate for **3 min**.

t. Proceed to Coverslipping.

Coverslipping

Before mounting the coverslip, gently flick the slide to remove any droplets and ensure it 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.

Use permanent, organic solvent-based mounting media to apply the coverslip. Consult the Atera Gene Expression Protocol Planner (CG001669) for tested part numbers.

a. Place slides on a flat, clean, nonabsorbent work surface.

b. Using a **wide-bore** pipette tip, add **150–200 µl** mounting media to uniformly cover all tissue sections on each 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 slides for **30 min** at **room temperature**.

f. Once coverslipping is complete, 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.**

2. Troubleshooting

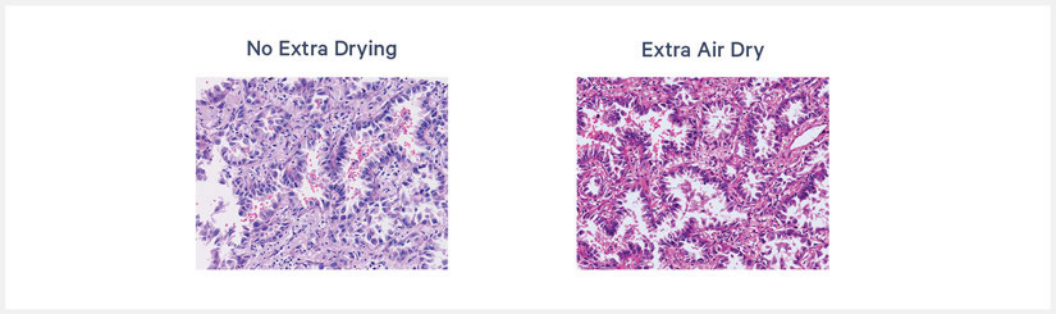
Dry Tissue

- Tissue shrinking is likely due to slides drying during the H&E staining workflow.

Tissue drying can occur when:

- Slides are not kept hydrated in between the Atera workflow and H&E staining.
- Slides are kept too long in ethanol during ethanol washes.

Using tap water vs. Milli-Q water will not impact slide drying during H&E staining.

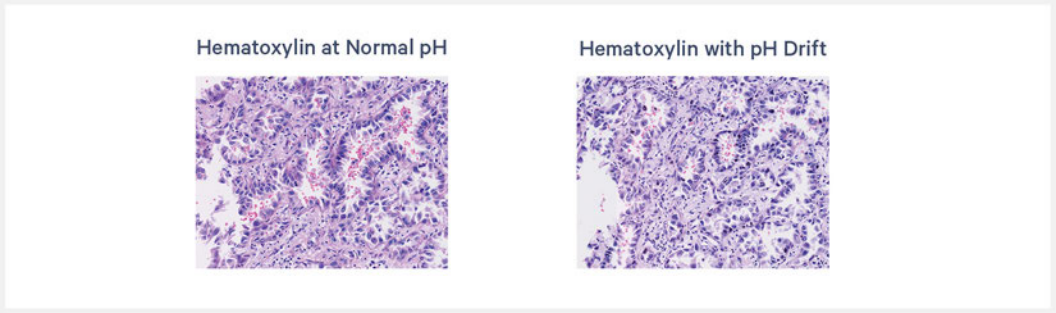


Protocol & Reagent Deviations

Staining outcome is highly sensitive to reagent quality, concentration, and incubation timing. Any deviation from the validated protocol — including substituting reagents, altering staining times, or using improperly prepared solutions — can significantly compromise results.

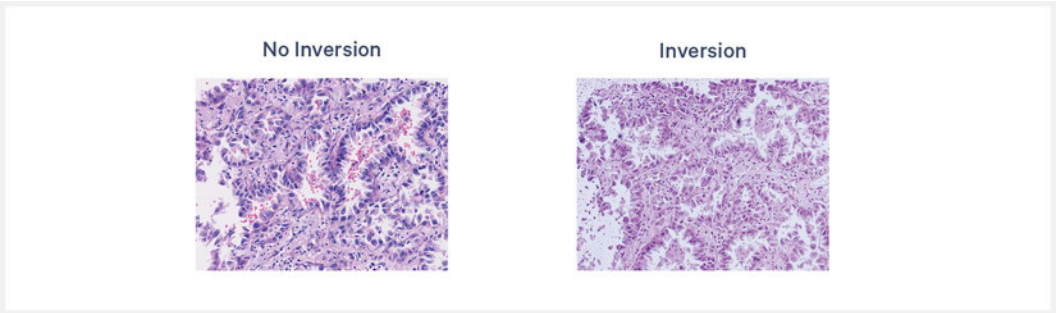
pH Drift in Hematoxylin

Hematoxylin solutions are slightly acidic and will shift over time, particularly if exposed to air, light, or alkaline carryover from rinse water. A basic pH tap water source can raise the pH of the hematoxylin, making it less effective, leading to pale or uneven nuclear staining. Solutions should be prepared fresh, filtered before use, and checked with a pH strip at the start of each run.



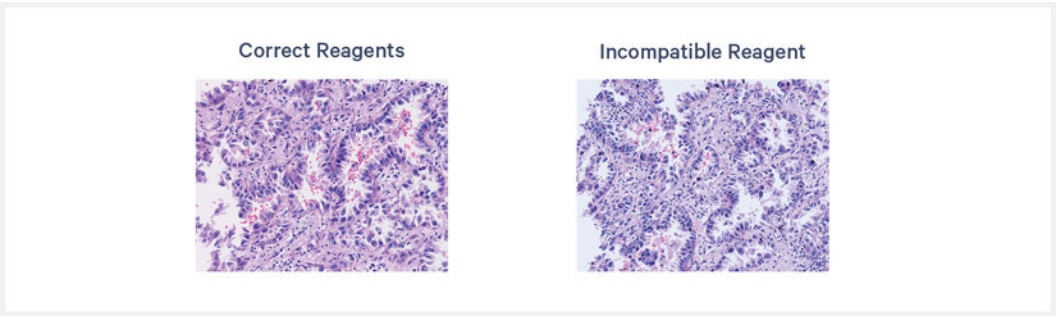
Hematoxylin & Eosin Inversion

Swapping hematoxylin and eosin solutions produces an extremely dark, near-black tissue section with no interpretable morphology. Tissue will be uniformly dark purple or near-black with no pink counterstaining. Verify reagent positions before beginning the run.



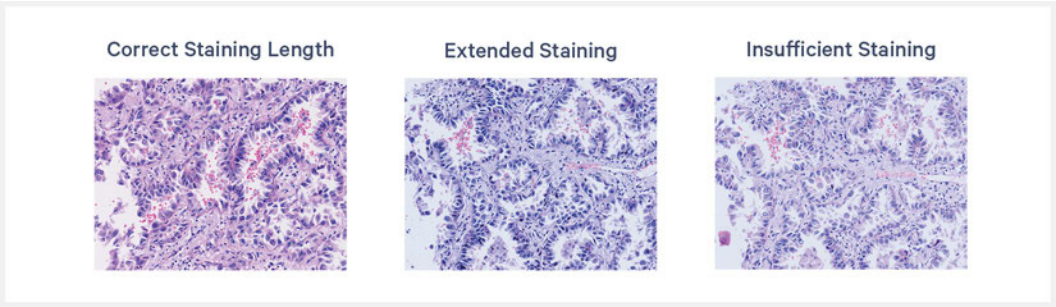
Incompatible Third-Party Reagents

Substituting validated hematoxylin, bluing reagents, or eosin formulations with alternatives from non-validated kits can cause cracking of the tissue section and a dark purple appearance. Only use reagent combinations validated for the Atera workflow.



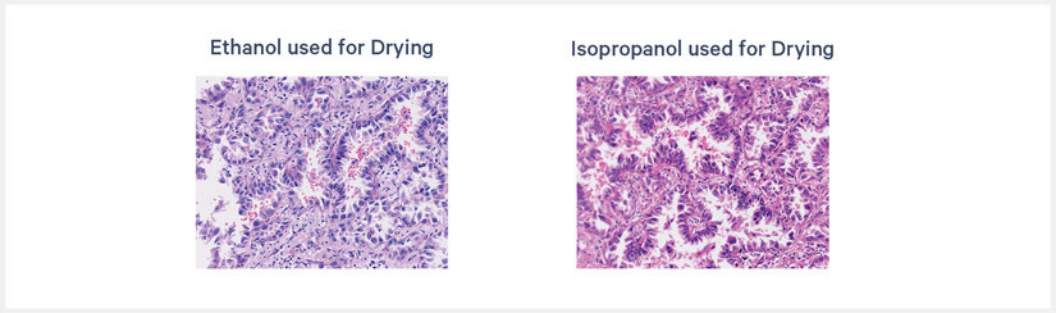
Prolonged or Insufficient Staining Times

If hematoxylin staining is too long, or if differentiation is inadequate, the nuclear stain becomes so dark that the chromatin pattern is lost and non-nuclear elements take up hematoxylin staining. Conversely, too short an incubation or over-differentiation yields pale, washed-out nuclei. If staining time in eosin is too long, or differentiation in the alcohols following eosin is inadequate, cytoplasmic staining becomes overly intense. The differentiation between collagen, smooth muscle, and red cells is lost. Adhere strictly to validated incubation times and differentiation steps.



Isopropanol vs. Ethanol

Isopropanol does not differentiate eosin in the same manner as ethanol. If ethanol is unavailable and isopropanol is substituted, eosin overstaining is likely. DO NOT substitute reagents.



Tissue Too Dark or Overstained Nuclei

A dark purple phenotype is possible, where nuclei are intensely stained to the point that chromatin patterns are unresolvable. This can be caused by reagent concentration swaps or reagent concentration errors.

Tissue Understained

Reagent age or pH can lead to understained tissue. For example, if nuclei appear tan, pink, or red instead of blue-purple, the hematoxylin may be overoxidized. Verify hematoxylin is within the expiration date. If the pH of the eosin solution is above 5.0 — possibly caused by carryover of the bluing reagent — the cytoplasmic stain will be pale. Check the pH of the eosin solution.

Incomplete rehydration prior to staining may also lead to suboptimal staining. Ensure slides are properly rehydrated after ethanol dehydration.

Tissue Shrinkage and Image Registration Misalignment

Tissue shrinkage may impact alignment between the Atera Instrument's spatial image registration and post-run H&E images. The result is a failure of nuclear overlap — cell boundaries and transcript calls no longer register correctly to the H&E morphology.

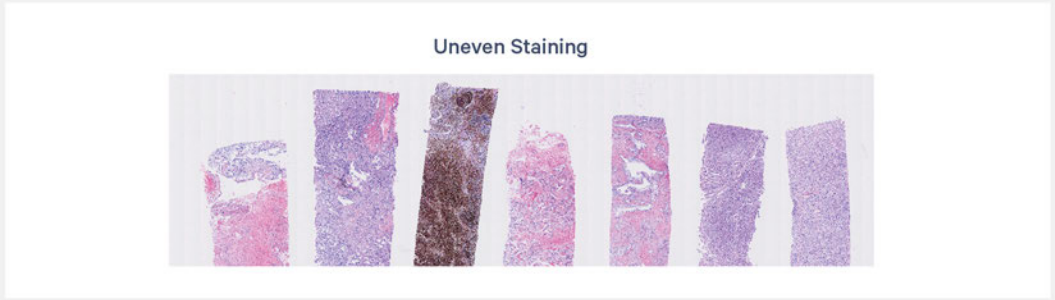
Tissue shrinkage is primarily caused by the tissue drying during a time that is not specified by the protocol. Slides must remain properly hydrated from the end of the Atera Instrument run through the start of H&E staining. DO NOT leave slides exposed to air for extended periods between steps.

Leaving slides in graded ethanol washes for longer than the specified times in the protocol can also cause progressive dehydration and shrinkage. Adhere strictly to stated timing for all dehydration steps and DO NOT leave slides unattended in ethanol stations.

Keep slides submerged in the appropriate buffer or reagent at all times when not actively being processed. If any interruption to the workflow is necessary, slides should be stored in PBS or the appropriate intermediate solution rather than left to air dry.

Uneven Staining

Sections that are too thin may result in pale eosin staining, due to less tissue available for the dye to bind. Conversely, sections that are too thick may stain too intensely or unevenly due to limited dye penetration through the full tissue depth. Unintentional tissue thickness may result from folds in the tissue section, resulting in locally darker staining. Document these sectioning artifacts, as they may confound morphological assessment.



Document Revision Summary

Document Number	CG001722
Title	Atera Gene Expression – Post-Atera H&E Staining Demonstrated Protocol
Revision	Rev A
Revision Date	Sept 2026
Specific Changes	Initial Release

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

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