Xenium In Situ Gene Expression - Post-Xenium Analyzer H&E Staining

Introduction

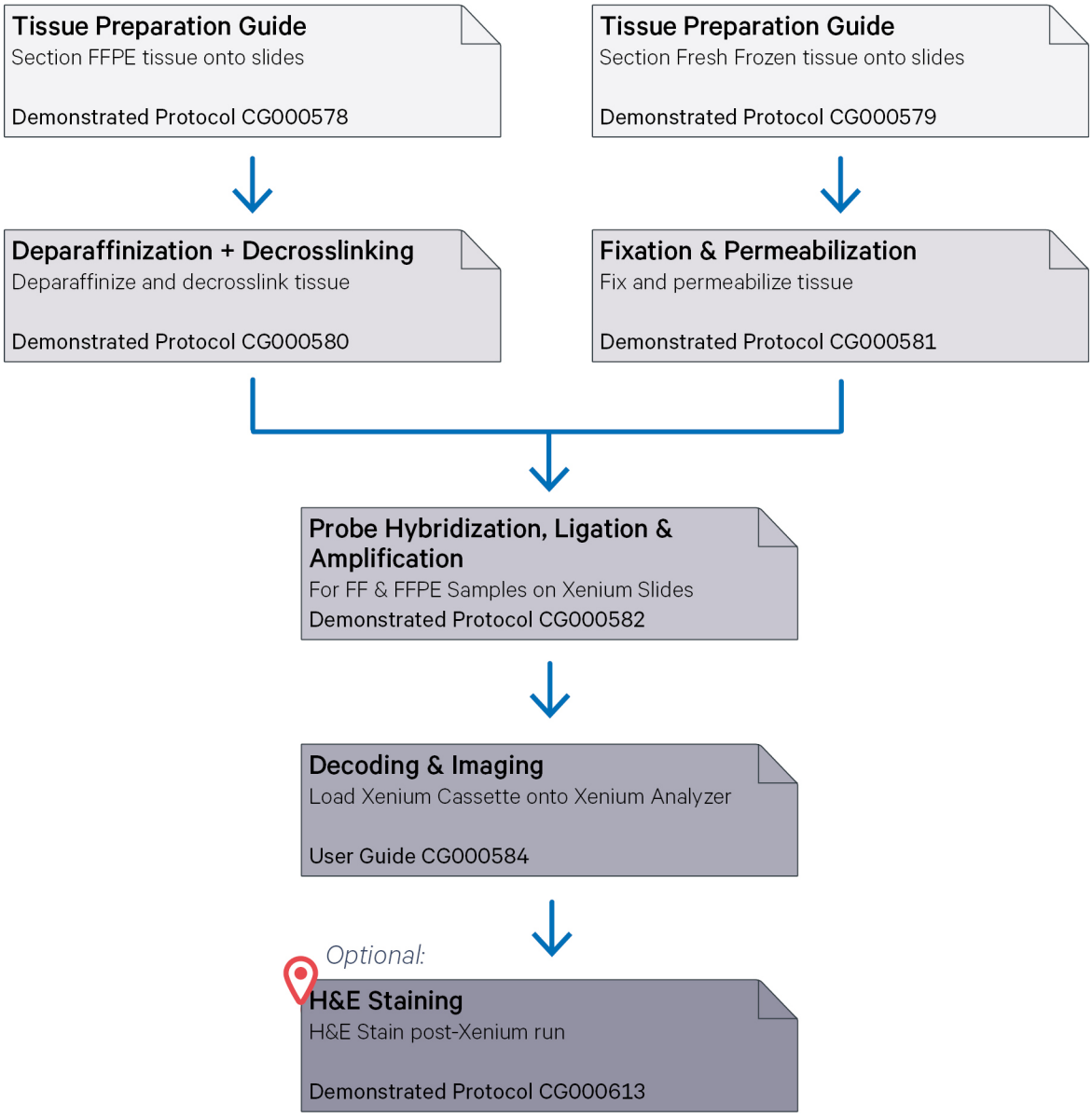
Xenium In Situ Gene Expression measures mRNA in formalin fixed & paraffin embedded (FFPE) and fresh frozen (FF) tissue sections that are placed onto Xenium Slides. Following a Xenium Analyzer instrument run, tissue sections on Xenium slides may be optionally processed for Hematoxylin & Eosin (H&E) staining. This document provides guidance for disassembly of the Xenium Cassette, removal of Autofluorescence Solution, and H&E staining steps for both FFPE and FF tissue sections. After Xenium Cassette Removal and Quencher Removal, follow the guidelines provided for Post-Xenium Analyzer H&E Staining or proceed to another staining protocol, if desired.

Additional Guidance

Proceed with the Post-Xenium Analyzer H&E Staining protocol following the Xenium Analyzer User Guide (CG000584) to obtain histological data that can be combined with gene expression data from the same tissue section. H&E Staining post-Xenium Analyzer instrument run is optional.

Contents

Workflow Overview



Visit the 10x Genomics Support website for the most current documentation.

Specific Reagents & Consumables

H&E Staining

The listed items have been tested by 10x Genomics and perform optimally with the assay. **Substituting materials may adversely affect system performance.** For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

For Quencher Removal

Item		Description	Vendor	Part Number
☐	Sodium hydrosulfite	Sodium hydrosulfite, technical grade	Sigma Aldrich	157953
☐	Forceps	Tweezers, 4" Wafer Handling	Excelta Corp	491P-SA-PI
☐	PBS *(optional)*	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
☐	Slide Mailers	Sim port Scientific LockMailer Tamper Evident Slide Mailer	Fisher Scientific	22-038-399

For H&E Staining

Item		Description	Vendor	Part Number
☐	Hematoxylin	Hematoxylin Solution, Mayer's	Sigma Aldrich	MHS16
☐	Eosin	Eosin Y Solution, Alcoholic	Leica	3801615
☐	Bluing Reagent	Bluing Solution	Dako	CS702
☐	Mounting Media	Surgipath SUB-X Mounting Media	Leica	3801741
☐	Ethanol	Ethyl Alcohol, 200 Proof, anhydrous	Millipore Sigma	E7023
		Ethanol absolute ≥99.5%, TechniSolv, pure (Europe)	VWR	83813.360DP
☐	Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
		Xylene, Histological Grade	Millipore Sigma	534056
☐	Forceps	Tweezers, 4' Water Handling	Excelta Corp	491P-SA-PI
☐	Filter Paper	Fisherbrand Qualitative Grade Plain Filter Paper Circles	Fisher Scientific	09-795-H
☐	Coverslips	Fisherbrand Cover Glasses: Rectangles	Fisher Scientific	12-544-EP
		Cover Glasses, Rectangles	VWR	16004-322

For H&E Staining	
	Additional Materials
☐	Vortex
☐	Staining jar/dishes
☐	Wide-bore pipette tips
☐	Ultrapure/Milli-Q Water *from Milli-Q Integral Ultrapure Water System or equivalent*

This list may not include some standard laboratory equipment.

Tips & Best Practices

Icons

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

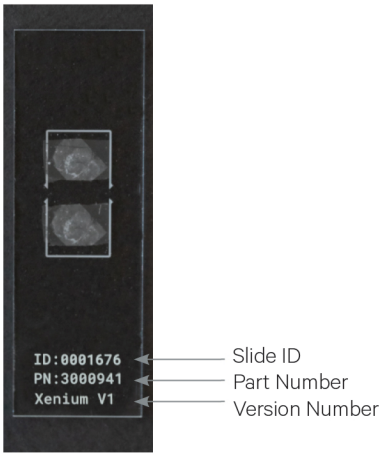
Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.

Xenium Slide Handling

- Always wear gloves when handling slides.
- The bottom of the slide is indicated by the etched label, which should be readable when in the proper position.
- Hold the slide on the label. DO NOT touch the tissue sections or near fiducials.
- Minimize exposure of the slides to sources of particles and fibers.

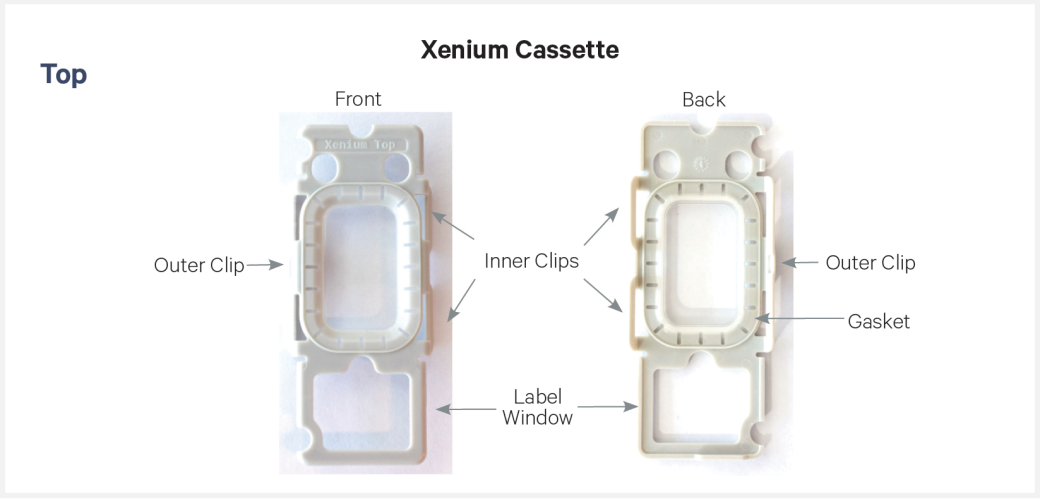
Xenium Slide with Tissue Sections



Slide Storage

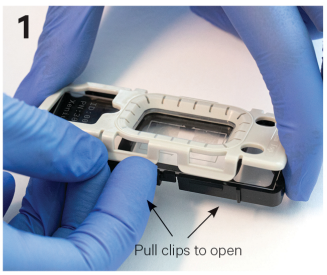
- Store analyzed Xenium Cassettes in PBS-T at 4°C following a Xenium Analyzer instrument run. Consult the Xenium Analyzer User Guide (CG000584) for more information about post-Xenium Analyzer storage conditions.
- Samples may be stored temporarily at 4°C post-H&E staining and imaged within 3 days after coverslipping.
- For long-term storage after imaging, maintain at room temperature in the dark.

Xenium Cassette

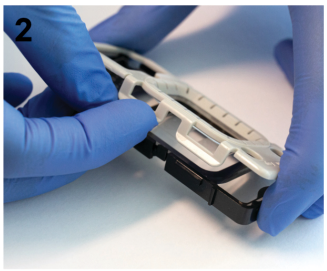


Xenium Cassette Removal

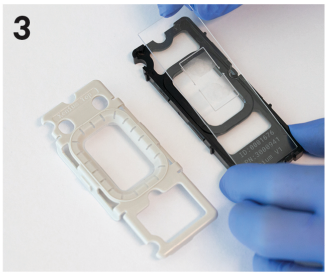
Pull inner clips from inner tabs to detach top and bottom halves of cassette



Open cassette by continuing to lift inner clips upward



Hold slide by the label and lift slide out from bottom half



Slides in images are representative.

Slide Incubation Guidance

Incubation at room temperature

- Place Xenium slide(s) with label toward the top of the slide mailer or coplin jar for incubations at room temperature.
- Separate multiple slides by at least one slotted channel inside the slide mailer.
- Avoid placing slides in the first or last slotted channel of the slide mailer or coplin jar. Slides with tissues in the first or last position may get scratched if facing the jar wall.
- Up to two slides can be processed per slide mailer and up to four slides can be processed per coplin jar. Note that buffer volume in slide mailers and coplin jars does not change if processing more than one slide.

Protocol Steps & Timing

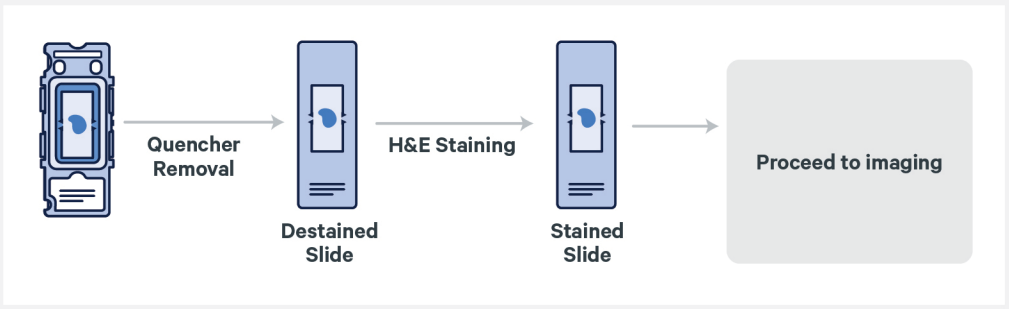
~2.5 h

Steps		Timing	Stop & Store
H&E Staining			
1.1	Buffer Preparation - Quencher Removal	15 min	
1.2	Cassette Removal	10 min	
1.3	Quencher Removal	15 min	STOP 4°C ≤ 2 days
1.4	Buffer Preparation - H&E Staining	15	
1.5	H&E Staining	1 h	
1.6	Coverslipping	35 min	STOP 4°C ≤ 3 days or room temperature (in the dark) long-term

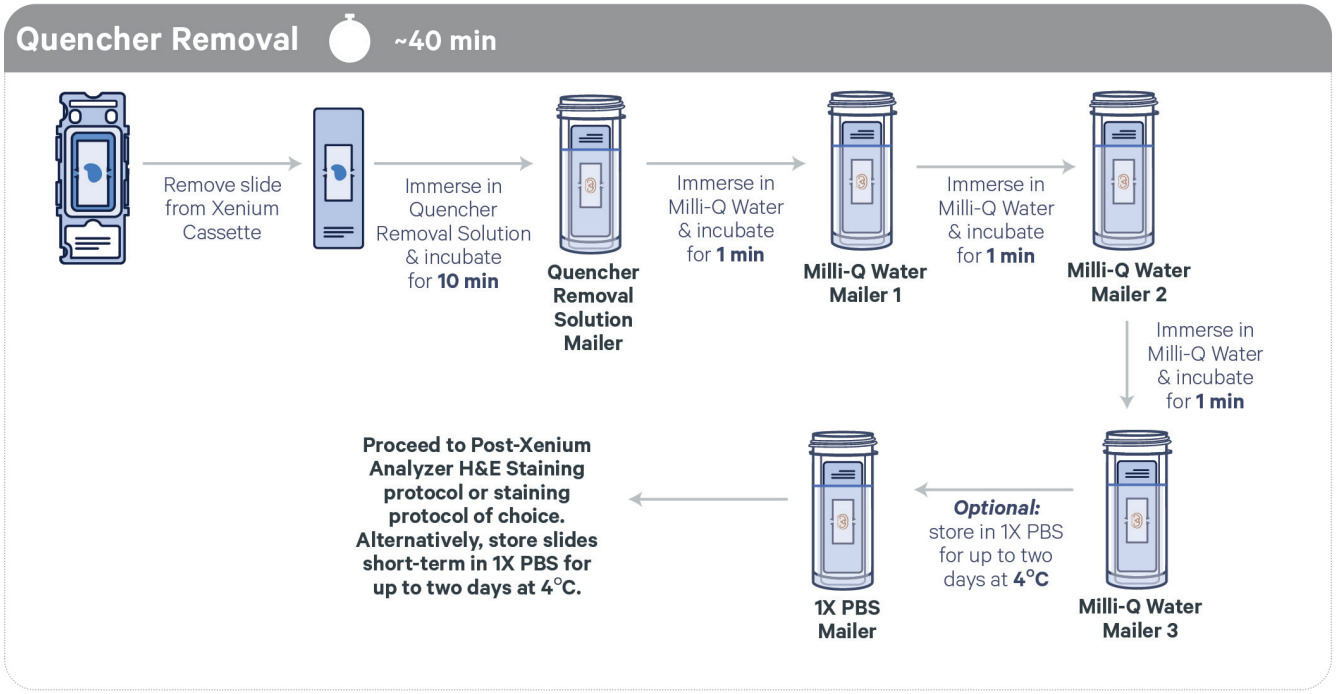
1. H&E Staining

1.0 Overview

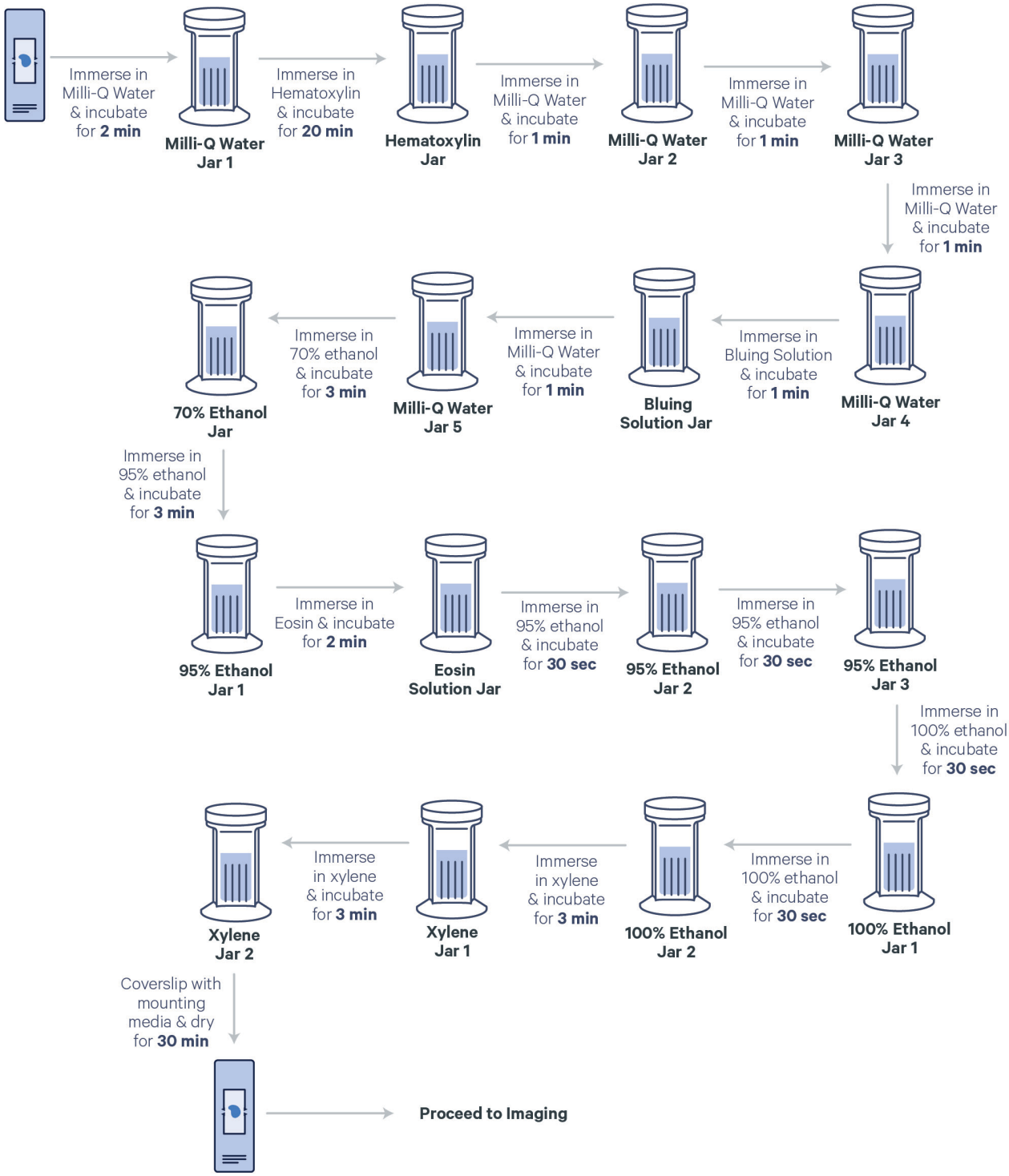
This chapter provides guidance on H&E staining of Xenium slides containing FFPE or fresh frozen tissue sections. First, Autofluorescence Solution is removed from tissue sections in a destaining step. Tissue sections are then stained in Hematoxylin and Eosin staining solutions and coverslipped to prepare for imaging. Proceed to imaging following the Post-Xenium Analyzer H&E Staining workflow.



Protocol Overview



H&E Staining ~2 h



Get Started - Quencher Removal

Quencher Removal Items		10x PN	Preparation & Handling	Storage
Obtain				
☐	Sodium hydrosulfite	-	-	Ambient
☐	Forceps	-	-	Ambient
☐	Slide Mailers	-	-	Ambient
☐	Milli-Q Water	-	-	Ambient
☐	PBS *(optional)*	-	-	Ambient
☐	Xenium Slides (2 pack) with FFPE or FF tissue sections	3000941	Retrieve from Xenium Analyzer and prepared according to Xenium Analyzer User Guide (CG000584).	4°C post Xenium-Analyzer run

1.1 Quencher Removal Preparation

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

a. Prepare Quencher Removal Solution **immediately** before use according to the table below.

Quencher Removal Solution must be prepared fresh before each use for optimal results. Prepare solution in a fume hood according to the instructions below due to the hazardous nature of sodium hydrosulfite.

Weigh 17.4 mg of sodium hydrosulfite in a fume hood and add to conical tube containing 10 ml Milli-Q Water. Cap tube and vortex 10 sec to dissolve. Vortexing can be performed outside of fume hood if tube is tightly capped. Maintain at room temperature.

Quencher Removal Solution				
Items		**Molecular Weight**	**Final**	**Total Amount (X units)**
☐	Milli-Q Water	-	-	10.0 ml
☐	Sodium Hydrosulfite	174.104 g/mol	10 mM	17.4 mg
☐	**Total**	**-**	**-**	**10.0 ml**

b. Prepare 1X PBS (optional) for storage of slides post-Quencher Removal. Add reagents in the order listed. Invert gently to mix. Maintain at room temperature.

1X PBS *(optional)*				
Items		**Molecular Weight**	**Final**	**Total Amount (ml)**
☐	Milli-Q Water	-	-	9.0
☐	PBS	10X	1X	1.0
☐	**Total**	**-**	**-**	**10.0**

c. Prepare four total slide mailers (and one optional mailer) for Quencher Removal steps.

For H&E Staining		
Items		**Preparation & Handling**
☐	Quencher Removal Solution	Label one slide mailer as Quencher Removal Mailer. Dispense 10 ml Quencher Removal Solution.
☐	Milli-Q Water	Label three slide mailers as Milli-Q Water Mailer 1, 2, and 3. Dispense 10 ml Milli-Q Water in each.
☐	1X PBS (optional)	Label one slide mailer as 1X PBS Mailer. Dispense 10 ml 1X PBS.

1.2 Cassette Removal

a. Retrieve the assembled Xenium Cassette containing FFPE or fresh frozen tissue sections from the Xenium Analyzer.

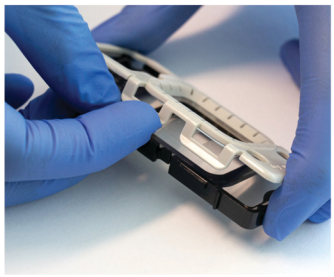
b. Using a pipette, remove all PBS-T from well corners.

c. Remove slide from Xenium Cassette.

TIPS

See Tips & Best Practices for guidance on Xenium Cassette Removal.

Remove Slide from Xenium Cassette

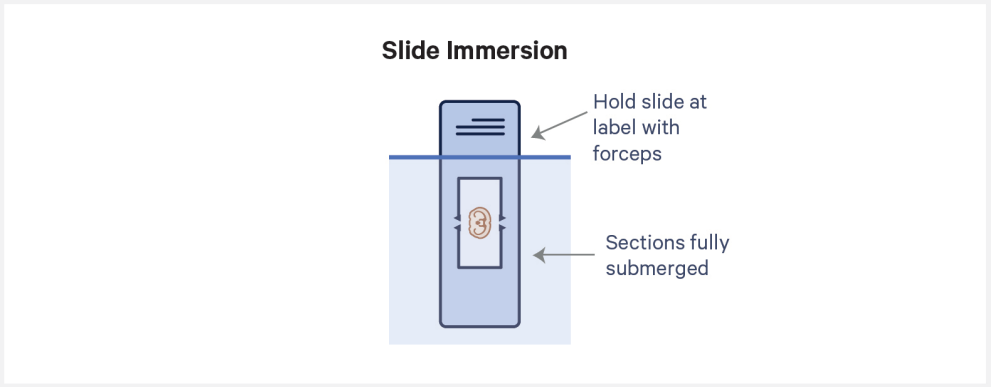


1.3 Quencher Removal

Quencher Removal steps should be performed in a fume hood due to the hazardous nature of sodium hydrosulfite. Quencher Removal is required for any staining protocol.

a. Gently immerse slide in the Quencher Removal Solution Mailer and incubate for **10 min** at **room temperature**.

When immersing slides, ensure that the tissue sections are completely submerged.



b. Gently immerse slide in Milli-Q Water Mailer 1 and incubate for **1 min** at **room temperature**.

c. Gently immerse slide in Milli-Q Water Mailer 2 and incubate for **1 min** at **room temperature**.

d. Gently immerse slide in Milli-Q Water Mailer 3 and incubate for **1 min** at **room temperature**.

STOP

e. Proceed to Post-Xenium Analyzer H&E Staining protocol on the following page or alternative staining protocol, if desired. Slides may be stored temporarily in a slide mailer containing 1X PBS at **4°C** for up to two days until ready to stain.

Get Started - H&E Staining

H&E Staining Items		10x PN	Preparation & Handling	Storage
Obtain				
☐	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

1.4 H&E Staining 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 sixteen 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 Mayer's 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 five coplin jars as Milli-Q Water Jar 1, 2, 3, 4, and 5. 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-resistent dishes, gloves, and forceps during workflow.*

1.5 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 **2 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 **20 min** at **room temperature**.

c. Gently immerse slide in the Milli-Q Water Jar 2 for **1 min** at **room temperature**.

d. Gently immerse slide in the Milli-Q Water Jar 3 for **1 min** at **room temperature**.

e. Gently immerse slide in the Milli-Q Water Jar 4 for **1 min** at **room temperature**.

f. Gently immerse slide in the Bluing Solution Jar for **1 min** at **room temperature**.

g. Gently immerse slide in the Milli-Q Water Jar 5 for **1 min** at **room temperature**.

h. Gently immerse slide in the 70% Ethanol Jar for **3 min** at **room temperature**.

i. Gently immerse slide in the 95% Ethanol Jar 1 for **3 min** at **room temperature**.

j. Gently immerse slide in the Eosin Solution Jar for **2 min** at **room temperature**.

k. Gently immerse slide in the 95% Ethanol Jar 2 for **30 sec** at **room temperature**.

l. Gently immerse slide in the 95% Ethanol Jar 3 for **30 sec** at **room temperature**.

m. Gently immerse slide in the 100% Ethanol Jar 1 for **30 sec** at **room temperature**.

n. Gently immerse slide in the 100% Ethanol Jar 2 for **30 sec** at **room temperature**.

o. Gently immerse slide in the Xylene Jar 1 for **3 min** at **room temperature**.

p. Gently immerse slide in the Xylene Jar 2 for **3 min** at **room temperature**.

1.6 Coverslipping

Prior to 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** mounting media to uniformly cover all tissue sections on the slide.

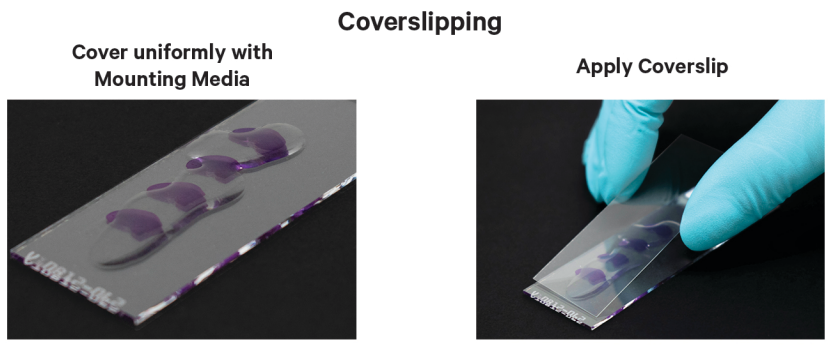
c. Apply the coverslip at an angle on one end of the slide. Slowly lower the coverslip, without introducing bubbles. Allow mounting media to spread and settle.

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**.

STOP

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.**



Slides in images are representative

Document Revision Summary

Document Number	CG000613
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