Xenium In Situ for FFPE – Deparaffinization & Decrosslinking

Introduction

Xenium In Situ for FFPE is designed to measure mRNA in tissue sections derived from formalin fixed & paraffin embedded (FFPE) tissue samples and requires a Xenium slide with intact tissue sections as input. This protocol outlines deparaffinization and decrosslinking of FFPE tissues for use with 10x Genomics Xenium protocols. Deparaffinized and decrosslinked tissue sections are inputs for these downstream Xenium workflows:

- Xenium In Situ Gene Expression (CG000582)
- Xenium In Situ Gene Expression with Cell Segmentation Staining (CG000749)
- Xenium Prime In Situ Gene Expression with optional Cell Segmentation Staining (CG000760)

Additional Guidance

Consult the Xenium In Situ for FFPE - Tissue Preparation Guide (Document CG000578) for complete information on sectioning FFPE tissue blocks and placing sections on Xenium Slides. Process the slides with the tissue sections as described in this protocol (CG000580). This protocol is compatible with both Xenium In Situ (referred to as Xenium v1) and Xenium Prime In Situ reagents and downstream assay workflows as specified in the table. Follow any specific deviations indicated in the protocol for Xenium v1 versus Xenium Prime.

Compatible Reagent Kits & Downstream Workflows		
	Xenium v1	**Xenium Prime**
Reagent Kits	Xenium Slides & Sample Prep Reagents PN-1000460	Xenium Prime Sample Preparation Reagents PN-1000720
	Xenium Decoding Consumables PN-1000487	Xenium Cassette Kit v2 PN-1000723
	Xenium Instrument Accessory Kit Module A PN-1000530	Xenium Thermocycler Adaptor v2 PN-1000739
	Xenium Cassette Kit PN-1000566	
Assay Workflow	Xenium In Situ Gene Expression (CG000582) Xenium In Situ Gene Expression with Cell Segmentation Staining (CG000749)	Xenium Prime In Situ Gene Expression with optional Cell Segmentation Staining (CG000760)

Items needed from each kit while executing this protocol are listed in the following sections.

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Xenium In Situ Gene Expression Reagent Kits

Compatible only with the following Xenium v1 workflows:

- Xenium In Situ Gene Expression (CG000582)
- Xenium In Situ Gene Expression with Cell Segmentation Staining (CG000749)

Refer to SDS for handling and disposal information

Xenium Slides & Sample Prep Reagents (2 slides, 2 rxns) PN-1000460

Xenium Slides & Sample Prep Reagents
(2 slides, 2 rxns), PN-1000460
Store at −20°C

	#	PN
Xenium Probe Hybridization Buffer	1	2000390
Xenium Post Hybridization Wash Buffer	1	2000395
Xenium Ligation Buffer	1	2000391
Xenium Ligation Enzyme A	1	2000397
Xenium Ligation Enzyme B	1	2000398
Xenium Amplification Mix	1	2000392
Xenium Amplification Enzyme	1	2000399
Reducing Agent B	1	2000087
Xenium Autofluorescence Mix	1	2000753
Xenium FFPE Tissue Enhancer*	1	2000798
Xenium Nuclei Staining Buffer	1	2000762
Perm Enzyme B	1	3000553
Xenium Slides (2 pack)	1	3000941

10x GENOMICS

Only Xenium FFPE Tissue Enhancer (PN-2000798) and Perm Enzyme B (PN-3000553) are needed for this workflow.

Xenium Decoding Consumables (1 Run, 2 Slides) PN-1000487

Xenium Decoding Consumables
(1 run, 2 slides), PN-1000487
Store at ambient temperature

		#	PN
	Xenium Cassette Kit (2 cassettes + 16 lids)	1	1000566
	Extraction Tip	1	2000757
	Pipette Tips	1	3000866
	Xenium Buffer Cap	4	3000949
	Xenium Objective Wetting Consumable	1	2000749
●	Deionized Water (bottle)	1	3001198
●	Xenium Sample Wash Buffer A (bottle)	1	3001199
●	Xenium Sample Wash Buffer B (bottle)	1	3001200
●	Xenium Probe Removal Buffer (bottle)	1	3001201

10x GENOMICS

Only the Xenium Cassette Kit (2 cassettes + 16 lids) (PN-1000566) is needed for this workflow.

Xenium Instrument Accessory Kit Module A PN-1000530

Xenium Instrument Accessory Kit Module A
PN-1000530
Store at ambient temperature

	#	PN
Waste Bottle	1	3000955
Xenium Waste Tip Tray	1	3000957
Xenium Thermocycler Adaptor	1	3000954

10x GENOMICS

Only the Xenium Thermocycler Adaptor (PN-3000954) is needed for this workflow.

Xenium Cassette Kit - (2 Cassettes) PN-1000566

Xenium Cassette Kit (2 cassettes)
PN-1000566
Store at ambient temperature

	#	PN
Xenium Cassette lids (16 ct)	1	3001046
Xenium Cassettes (2 pack)	1	3000951

10x GENOMICS

Purchase the Xenium Cassette Kit (2 cassettes) (PN-1000566) for additional cassettes as needed.

Xenium Prime In Situ Gene Expression Reagent Kits

Compatible only with the following Xenium Prime workflows:

- Xenium Prime In Situ Gene Expression with optional Cell Segmentation Staining (CG000760)

Refer to SDS for handling and disposal information.

Xenium Prime Sample Preparation Reagents - (2 rxns) PN-1000720

Xenium Prime Sample Prep Reagents, Module A
(2 rxns), PN-1000720
Store at –20°C

	#	PN
Perm Enzyme B	1	3000553
FFPE Tissue Enhancer*	1	2000798
Priming Hyb Buffer	1	2001228
Post-Priming Wash Buffer	1	2001229
RNase Enzyme	1	3000593
2X RNase Buffer	1	2000411
Polishing Enzyme	1	2001230
Polishing Buffer	1	2001231
Probe Hyb Buffer B	1	2001232

10x GENOMICS

Only FFPE Tissue Enhancer (PN-2000798) and Perm Enzyme B (PN-3000553) are needed in this workflow.

**The reagent name may or may not include the prefix "Xenium"; Irrespective of the prefix, the indicated part number is associated with the reagent name.*

Xenium Cassette Kit v2 - (2 cassettes) PN-1000723

Xenium Prime Cassettes and Inserts
PN-1000723
Store at ambient temperature

	#	PN
Xenium Cassette Top v2	2	3002205
Xenium Cassette Bottom v2	2	3002223
Xenium Cassette Lid v2	8	3002206
Xenium Cassette Insert	4	3001885

10x GENOMICS

Xenium Cassette Insert is not needed in this workflow.

Xenium Thermocycler Adaptor v2- (1 adaptor) PN-1000739

Recommended Thermal Cyclers

Xenium v1 validated thermal cyclers:

Supplier	Description	Part Number
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module (discontinued)	1851197
Analytik Jena	Biometra TAdvanced 96 SG with 96-well block (silver, 0.2 mL) and gradient function	846-x-070-241 *(where x=2 for 230 V; 4 for 115 V; 5 for 100 V, 50-60 Hz)*
VWR	Gradient thermal cycler, XT^{96} Gradient, with 96-well gradient block and standard lid	76452-153
Marshall Scientific	MJ Research PTC-200 Thermal Cycler (discontinued)	05434-05

Xenium Prime validated thermal cyclers:

Supplier	Description	Part Number
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module (discontinued)	1851197
Bio-Rad	PTC Tempo Deepwell Thermal Cycler	12015392
Analytik Jena	Biometra TAdvanced 96 SG with 96-well block (silver, 0.2 mL) and gradient function	846-x-070-241 *(where x=2 for 230 V; 4 for 115 V; 5 for 100 V, 50-60 Hz)*
ThermoFisher Scientific	VeritiPro 96-well Thermal Cycler	A48141

Specific Reagents & Consumables

FFPE Tissue Sections: Deparaffinization & Decrosslinking

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 FFPE Tissue Sections: Deparaffinization & Decrosslinking				
Item		**Description**	**Vendor**	**Part Number**
☐	Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
	or	Xylene, Histological Grade	Millipore Sigma	534056
	Neo-clear	Neo-clear Xylene Alternative Substitute *Only tested for the Xenium Gene Expression workflow*	Millipore Sigma	1098435000
☐	Ethanol	Ethyl Alcohol, 200 Proof, anhydrous	Millipore Sigma	E7023
		Ethanol absolute ≥99.5%, TechniSolv, pure (Europe)	VWR	83813.360DP
☐	Nuclease-free Water	Nuclease-free Water (not DEPC-treated)	Thermo Fisher Scientific	AM9932/ AM9937
☐	PBS	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
☐	Urea	Urea Solution, 8M	Millipore Sigma	51457
☐	10% Tween-20	Tween 20 Surfact-Amps Detergent Solution (10% solution) *(not 100% Tween diluted to 10%)*	Thermo Fisher Scientific	28320
		10% Tween-20	Bio-Rad	1662404
☐	Perm Enzyme B	Perm Enzyme B	10x Genomics	3000553
☐	Xenium FFPE Tissue Enhancer	Xenium FFPE Tissue Enhancer	10x Genomics	2000798
☐	Forceps	Tweezers, 4" Wafer Handling	Excelta Corp	491P-SA-PI
☐	Staining jar/dishes	Coplin Jar	VWR	100500-232
		Staining Dishes	VWR	25608-906
☐	Section dryer oven	Epredia High Capacity Section Dryer *(Or equivalent. Thermal cycler may also be used for section drying).*	Fisher Scientific	A84600051
	Additional Materials			
☐	Water bath or Thermomixer with 2 ml adapter			

For FFPE Tissue Sections: Deparaffinization & Decrosslinking	
☐	Thermal Cycler *(see Recommended Thermal Cyclers)*
☐	Slide drying rack
☐	Fume Hood
☐	Vortex

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

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.

Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.

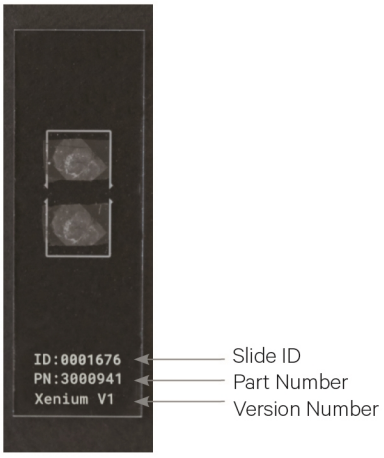
The instructions apply to both Xenium v1 and Xenium Prime assays. The consumables are referred to generically without always citing the version suffix. Based on the assay choice, always ensure that either Xenium v1 or Xenium Prime specific reagents and consumables are used.

Xenium Slide

- Xenium slides include an imageable area outlined by a white line measuring 12 mm x 24 mm, with an available sample positioning area measuring 235 mm^2 (10.45 mm x 22.45 mm). The available sample positioning area will be referred to as the Sample Area for the remainder of this document.
- The Sample Area is surrounded by fiducials. Tissue sections are placed within the Sample Area without obstructing the fiducials. The imageable area includes the area within the fiducial frame + Sample Area.
- The Sample Area can accommodate as many tissue sections as can fit within the space. Ensure tissue sections (including wax) DO NOT overlap.
- An etched label denoting the Slide ID, Part, and Version numbers is located at the bottom of the slide. Tissue sections should be placed on labeled-side of slide.

  The Xenium Slide (PN-3000941) may not always include the suffix v1 in the etched label.

Xenium Slide with Tissue Sections



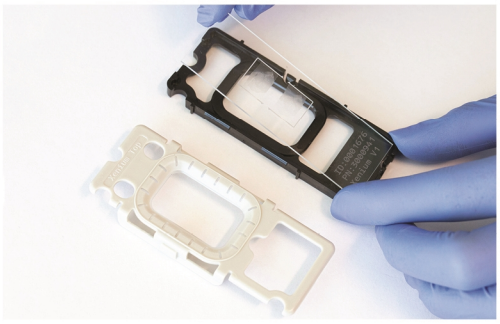
Xenium Slide Handling

- 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.
- The tissue sections should always be placed within the Sample Area on etched label side of the slide.
- 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.
- Keep the slide cassette 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. Avoid pipetting directly onto the tissue.
- After aspirating reagent from a slide, pipette new reagent onto same slide before moving onto aspiration of second slide.

The Xenium Slide (PN-3000941) may not always include the suffix v1 in the etched label.

The instructions apply to both Xenium Cassette and Xenium Cassette v2. The image shows a Xenium Cassette.

Xenium Slide Handling



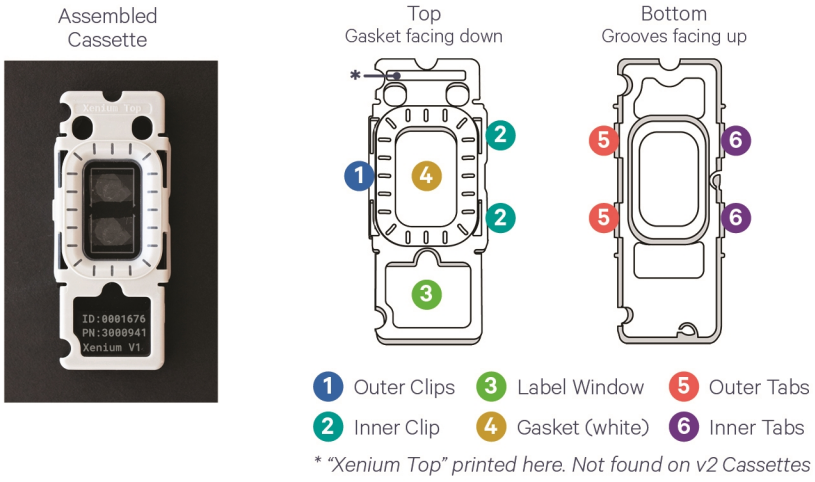
Note the Slide ID and Part numbers on the slide label will be required for downstream analysis.

Slide Storage

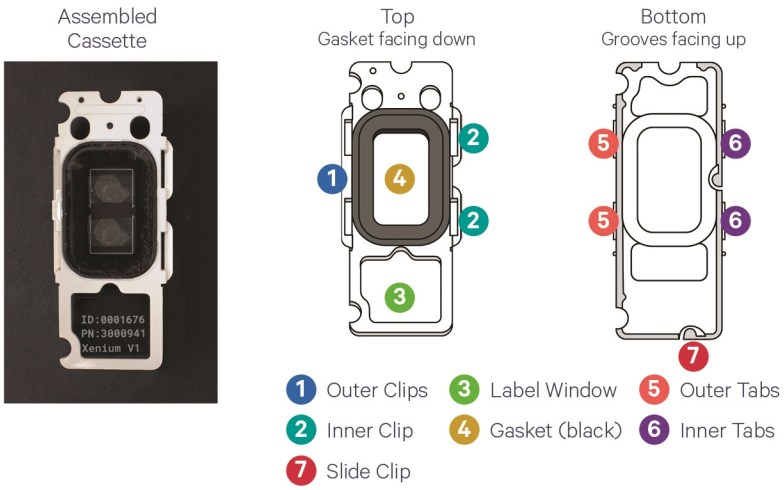
- Always store unused slides at -20°C in their original packaging and keep sealed. Once opened, slides should remain at room temperature in a desiccator and be used within one week to place tissue sections.
- After tissue sections are placed on the slide, store slides at room temperature in a desiccator for up to four weeks (not in a sealed container).

Xenium Cassette

Xenium Cassette
(Use with Xenium v1 Assays)



Xenium Cassette v2
(Use with Xenium Prime Assays)



The following guidance applies to both Xenium Cassette and Xenium Cassette v2

- The Xenium Cassette is a single use item.
- The Xenium Cassette encases the slide and creates a leakproof well for adding reagents.
- Place the slide in the Xenium Cassette only when specified.
- Inner and outer tabs on the bottom half of the Xenium Cassette are used for holding the slide in the cassette. Applying excessive force to the cassette may cause the slide to break.
- The Xenium Cassette is assembled manually. See Xenium Cassette Assembly & Removal instructions for details.
- The Xenium Cassette includes an attached Xenium Gasket. The Xenium Gasket corresponds to the Sample Area on the slides.
- The etched slide label is visible in the label window when properly assembled.
- Ensure that the Xenium Cassette and gasket are free of debris before assembly. If placing the top half of the cassette on a surface, ensure the gasket faces up so it does not collect debris.
- Visually inspect the gasket to ensure it is seated properly. If the gasket appears warped, the Xenium Cassette is safe to use if the cassette can fully close and no reagent leakage is observed.

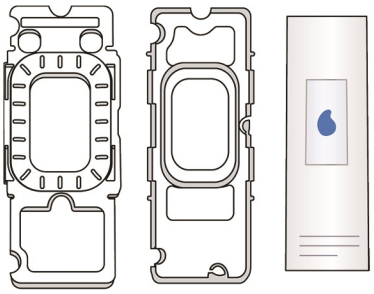
Xenium Cassette Assembly

Visually inspect cassette and gasket before cassette assembly.

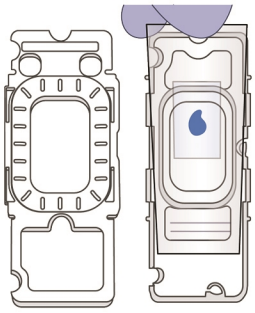
Xenium Cassette Assembly (for Xenium v1)

Exercise caution when handling slide edges to prevent injury.

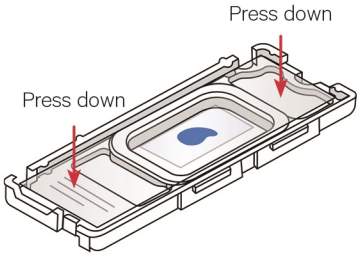
1 Place top and bottom halves of cassette on bench with the top cassette facing down and bottom cassette facing up.



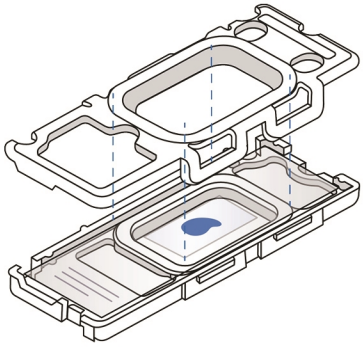
2 Place Xenium slide with tissue side facing upwards into bottom half of cassette; ensure label is toward bottom of cassette.



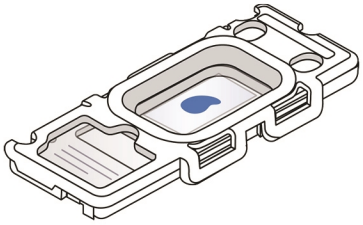
3 Press slide down into grooves of the bottom half of the cassette until it sits firmly in place.



4 Secure clips of top half with tabs of bottom half (on both sides).



5 Apply even pressure on top of cassette until all clips click shut. Verify that clips are completely secured over tabs.



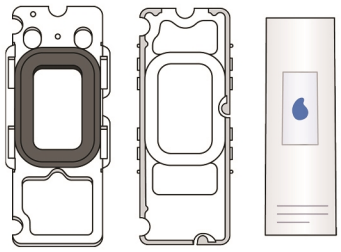
TIPS

Once cassette is assembled, DO NOT remove slide until after Xenium Analyzer imaging and decoding for optional H&E staining step.

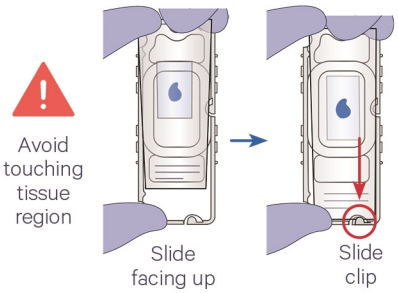
Xenium Cassette v2 Assembly (for Xenium Prime)

Exercise caution when handling slide edges to prevent injury.

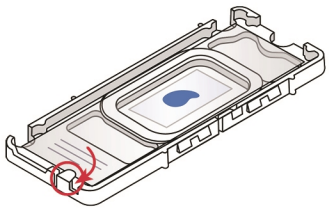
1 Place top and bottom halves of cassette on bench with the top cassette facing down and bottom cassette facing up.



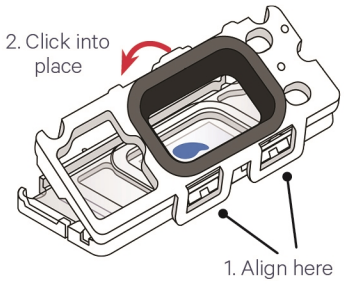
2 Place Xenium slide with label at bottom and tissue facing up into bottom half of cassette. Slip slide under slide clip located at bottom right of cassette.



3 After slide is safely underneath slide clip, place opposite side down into carrier. Ensure slide is sitting flat.

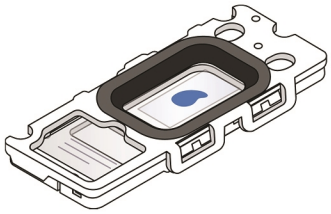


4 Align inner clips of top cassette to inner tabs of bottom cassette. Bring opposite side down until 2 audible clicks are heard.



5 Verify that clips are completely secured over tabs. Top cassette should sit flat and no gaps should be visible around entire cassette.*

**Consult Xenium Prime In Situ Gene Expresssion with optional Cell Segmentation Staining User Guide (CG000760) for cassette assembly failures.*

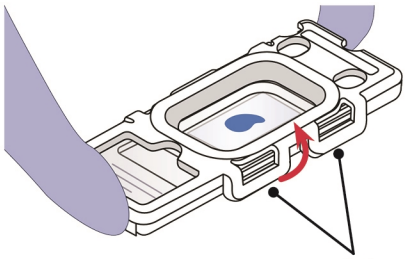


Xenium Cassette Removal

Xenium Cassette Removal (for Xenium v1)

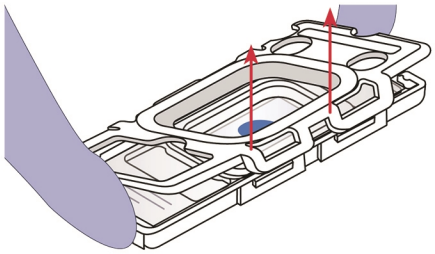
Exercise caution when handling slide edges to prevent injury.

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

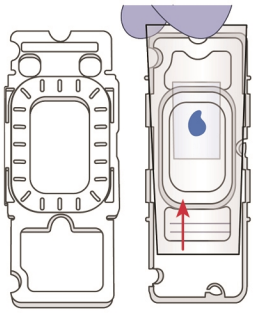


Pull clips

2 Open cassette by continuing to lift inner clips upward.



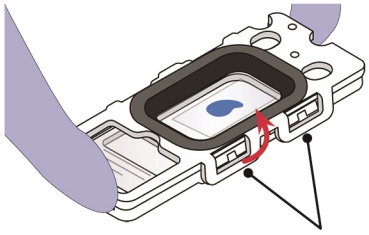
3 Hold slide by the label and lift slide out from bottom half.



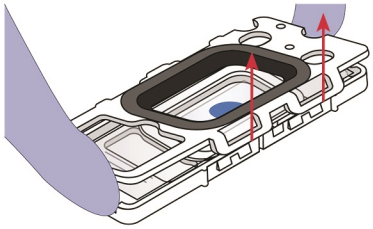
Xenium Cassette v2 Removal (for Xenium Prime)

Exercise caution when handling slide edges to prevent injury.

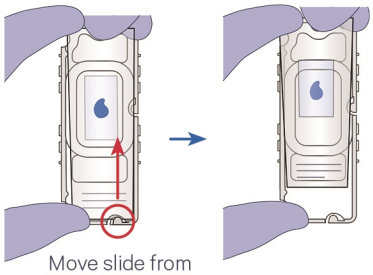
1 Pull inner clips from inner tabs to detach top and bottom halves of cassette.



2 Open cassette by continuing to lift inner clips of top cassette upward.

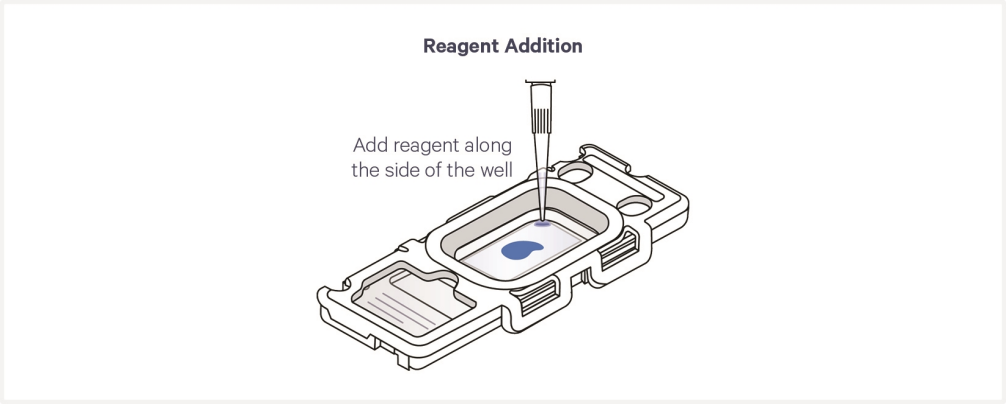


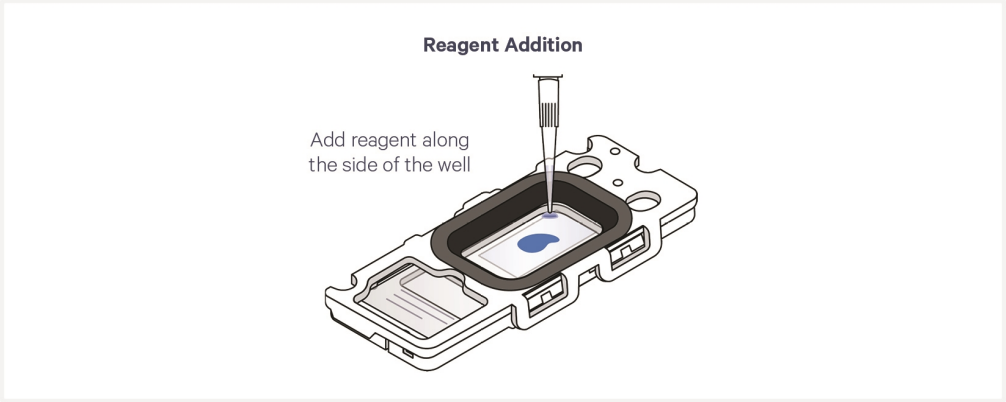
3 Hold top of slide and slowly lift slide out from underneath the slide clip. Then slowly remove from bottom cassette



Reagent Addition to Wells

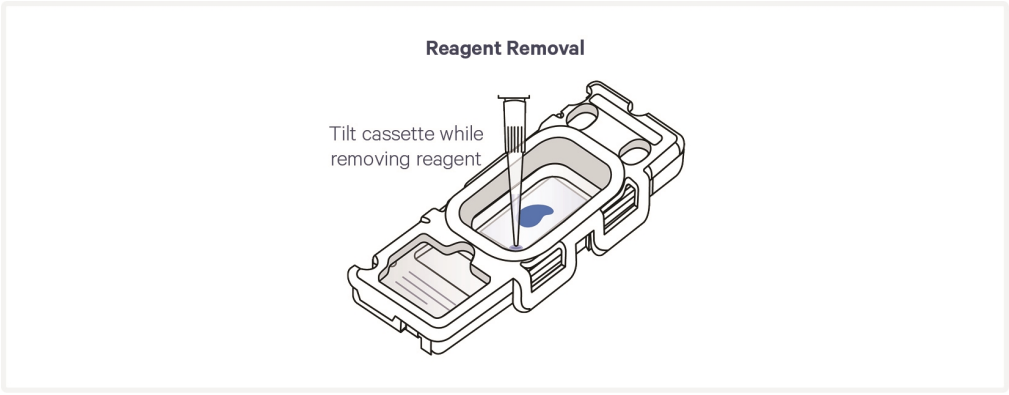
- Place assembled cassette flat on a clean work surface.
- Dispense and remove reagents along the side of the well without touching the tissue sections and without introducing bubbles.
- Always cover the Sample Area completely when adding reagents to the well. A gentle tap may help spread the reagent more evenly.

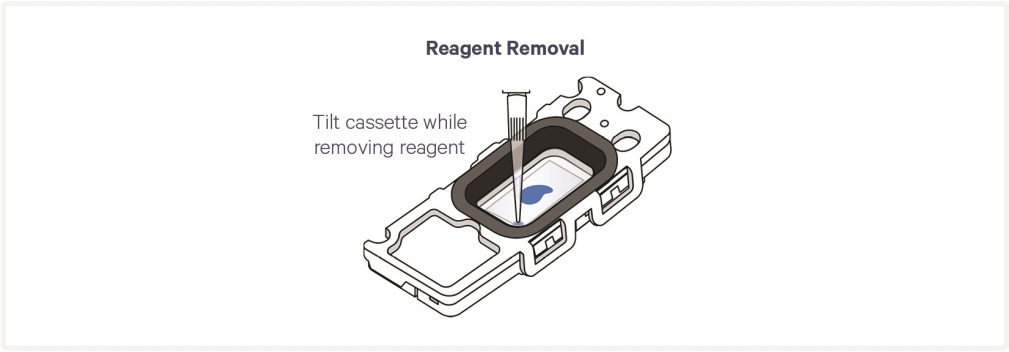




Reagent Removal from Wells

- Place assembled cassette flat on a clean work surface.
- Slightly tilt the cassette while removing the reagent.
- Place the pipette tip on the bottom edge of the well.
- Remove reagents along the side of the well without touching the tissue sections.
- Remove all liquid from the well in each step.





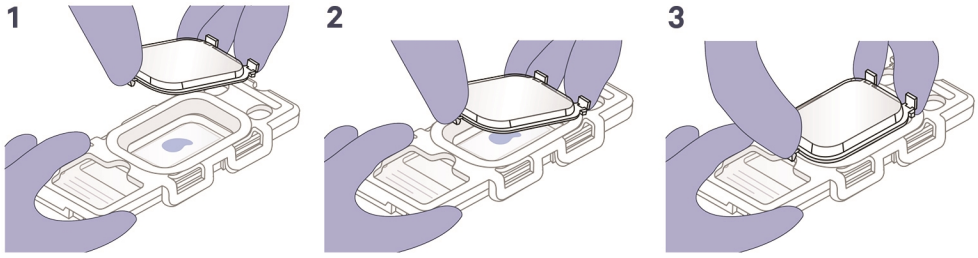
Xenium Cassette Lid Application & Removal

- Work on a clean surface.
- Use a new lid or reapply a used lid based on the instructions provided for a specific protocol step.

When handling an assembled cassette with the lid applied, always hold from the bottom of the cassette and not the lid.

Application

Applying the Xenium Cassette Lid

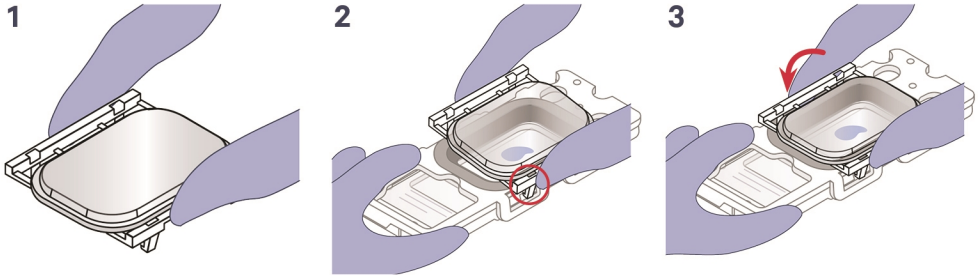


Hold the Xenium Cassette Lid with index and middle finger on two upper tabs and thumb on the lower clip.

Align lid with the surface of the Xenium Cassette. Hook the two upper clips into the two holes on the top of the cassette.

Push the lid down until the lower clip clicks into place. Inspect the lid to confirm placement.

Applying the Xenium Cassette Lid v2



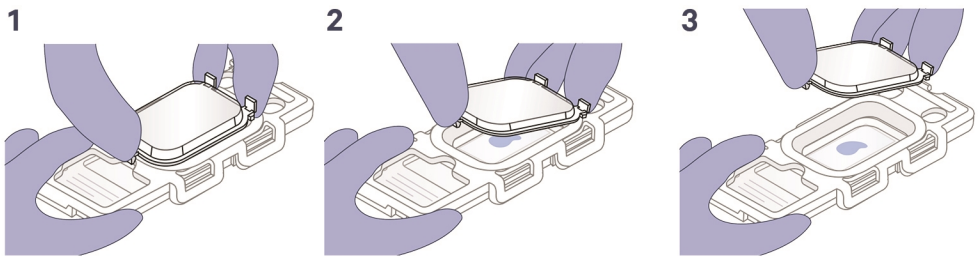
Hold the Xenium Cassette Lid v2 with thumb and index finger on the long sides.

On one side, align lid the lid feet to the notches on the cassette clip.. Hook the feet into the two side holes of the cassette.

Slowly push down on the other side of the lid until that side clicks into place. Inspect the lid to confirm placement.

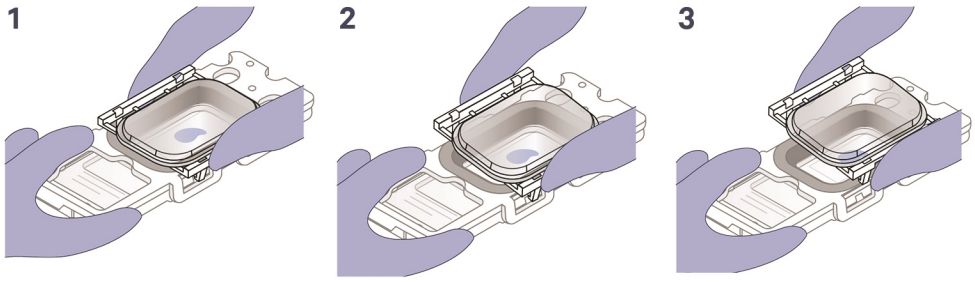
Removal

Removing the Xenium Cassette Lid



1. Push on the top of the two upper tabs with index and middle fingers.
2. Use thumb to push in on the lower clip. While maintaining inward pressure, pull upward with thumb to disengage lower clip.
3. Lift cassette up slowly to ensure no liquid splashes out of the well

Removing the Xenium Cassette Lid v2



1. Apply even pressure on both sides of the lid using thumb and index finger.
2. While maintaining inward pressure, pull upward with index finger to unhook the side feet from the cassette.
3. Slowly lift cassette lid up to unhook the remaining lid feet. Ensure no liquid splashes out of the well

Xenium Cassette Lids are a single use item and should be discarded after each use unless otherwise indicated. PBS-T washes DO NOT require sealing of the cassette with a lid.

- When saving the lid is specified, wipe it with a lint-free laboratory wipe, place on a clean surface, and reuse in the next indicated step.

Slide Incubation Guidance

Incubation at a specified temperature

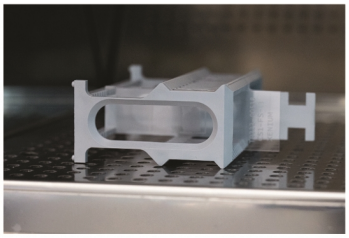
The following guidance applies to:

- Thermal Cycler Adaptor & Xenium Cassette (Xenium v1)
- Thermal Cycler Adpator v2 & Xenium Cassette v2 (Xenium Prime)

Incubation using a Section Dryer Oven:

- Place the slides in a slide drying rack sideways to minimize paraffin wax entering neighboring tissue.
- Close the lid when incubating the slide in the oven.

Place in a Rack



The instructions apply to both Thermocycler Adaptor and Thermocycler Adaptor v2. The illustrations show a Thermocycler Adaptor.

Incubation using a Thermal Cycler:

- Position a Xenium 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 Thermocycler Adaptor is fully inserted into the thermal cycler and is in contact with the thermal cycler block surface uniformly.
- When incubating a slide, position the slide on the Thermocycler Adaptor with the tissue side facing up.
- Ensure the Sample Area is aligned with the corresponding area on the Thermocycler Adaptor. DO NOT close lid.
- When incubating a slide encased in a Xenium Cassette, place the assembled unit on the Thermocycler Adaptor with the well facing up. The cassette

should always be sealed with a Xenium Cassette Lid when on the Thermocycler Adaptor unless indicated otherwise.

Tightening the thermal cycler lid

- Thermal cycler lid contact with the Xenium Cassette Lid is critical for assay performance.
- Tighten the thermal cycler lid until an audible click is heard.
- Tightening past the click risks breaking the slide.

Incubation at room temperature

- Place the slide/Xenium Cassette on a flat, clean, non-absorbent work surface.
- Ensure that no absorbent surface is in contact with the reagents on the slide during incubation.

Processing a Single Xenium Slide

The following guidance applies to both Xenium v1 and Xenium Prime workflows.

- Xenium reagent kits are sufficient for two reactions, and for optimal Xenium Analyzer throughput, two slides should be run at the same time.
- When pairing Xenium v1 or Xenium Prime workflows with Cell Segmentation Staining, process two slides per run. The Xenium Stain Enhancer (PN-2000992) cannot be stored once resuspended. Therefore, the 10x reagents for Xenium v1 and Xenium Prime Cell Segmentation workflows can only be used for one round, regardless of whether one or two slides are processed in a single run.

- It is possible to perform the Xenium workflow with a single slide. To do this, ensure the following best practices are followed for optimal assay performance:
  - Assemble a mock Xenium Cassette using a blank slide and a cassette from the Xenium Cassette Kit.
  - Insert the blank slide into the Xenium Cassette. Cassettes should be assembled following the instructions in Tips & Best Practices for Xenium Cassette Assembly.
  - Attach a lid from the Xenium Cassette Kit to the cassette containing the blank slide following Tips & Best Practices for Xenium Lid Application. It is not necessary to add liquid to the slide well before adding the lid.
  - For all incubation steps with the thermal cycler lid closed, ensure the mock slide cassette is placed alongside the Xenium Cassette containing tissue on the Thermocycler Adaptor.

Tissue Detachment on Xenium Slides

- Monitor section adhesion on Xenium slides throughout the workflow.
- Tissue detachment during the workflow can negatively impact performance. If observed, contact support@10xgenomics.com.
- For more information, refer to Troubleshooting.

Protocol Steps & Timing

~4.5 hours*

Steps		Timing	Stop & Store
Deparaffinization & Decrosslinking			
1.1	Buffer Preparation - Deparaffinization & Decrosslinking	30 min	
1.2	Deparaffinization	• Xenium v1 - 3 h (includes 2 h incubation at 60°C) • Xenium Prime - 1.5 h (includes 30 min incubation at 60°C)	
1.3	Cassette Assembly	10 min	
1.4	Decrosslinking	1 h	
*If using Xenium Prime reagents, the total protocol time is ~3 h			

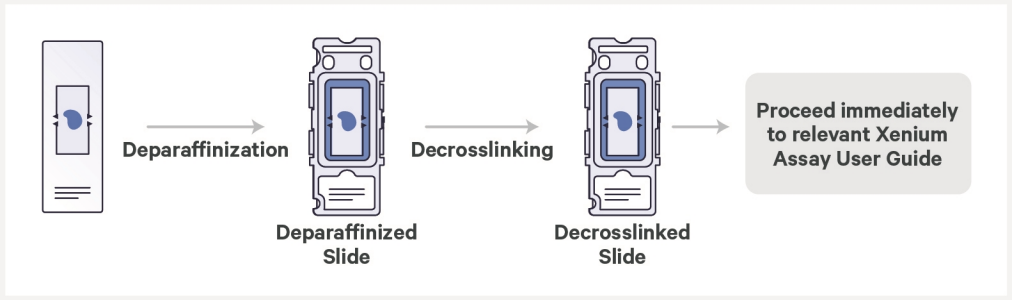
Note there are no safe stopping points during this workflow.

1. Deparaffinization & Decrosslinking

1.0 Overview

This chapter provides guidance on deparaffinization and decrosslinking of Xenium slides containing FFPE tissue sections that are dried overnight in a desiccator. After paraffin is removed from the tissue sections during the deparaffinization process, tissues are rehydrated and decrosslinked to release the sequestered RNA from the tissue. Following deparaffinization and decrosslinking, proceed immediately to the relevant downstream assay user guide:

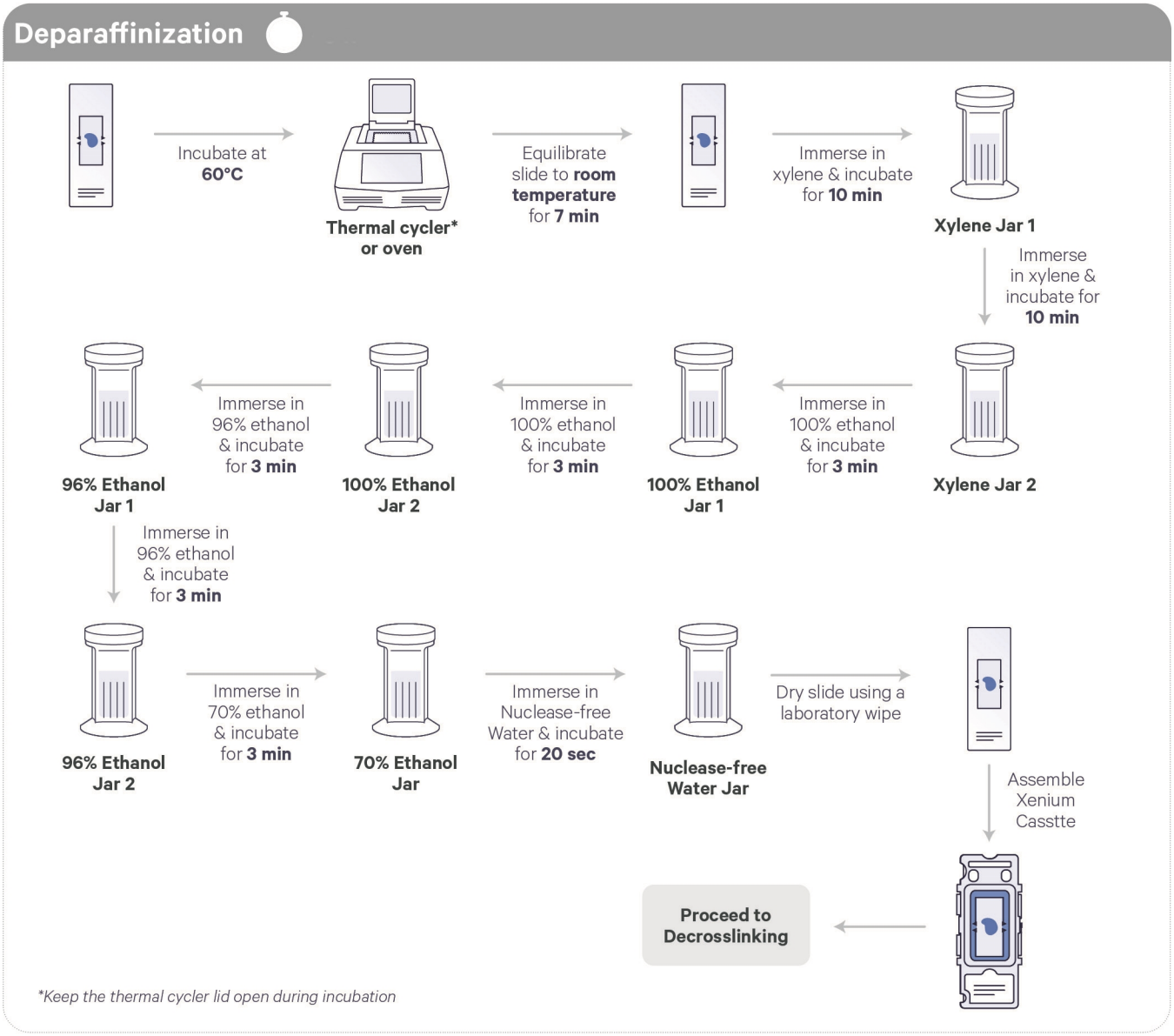
- Xenium In Situ Gene Expression (CG000582)
- Xenium In Situ Gene Expression with Cell Segmentation Staining (CG000749)
- Xenium Prime In Situ Gene Expression with optional Cell Segmentation Staining (CG000760)



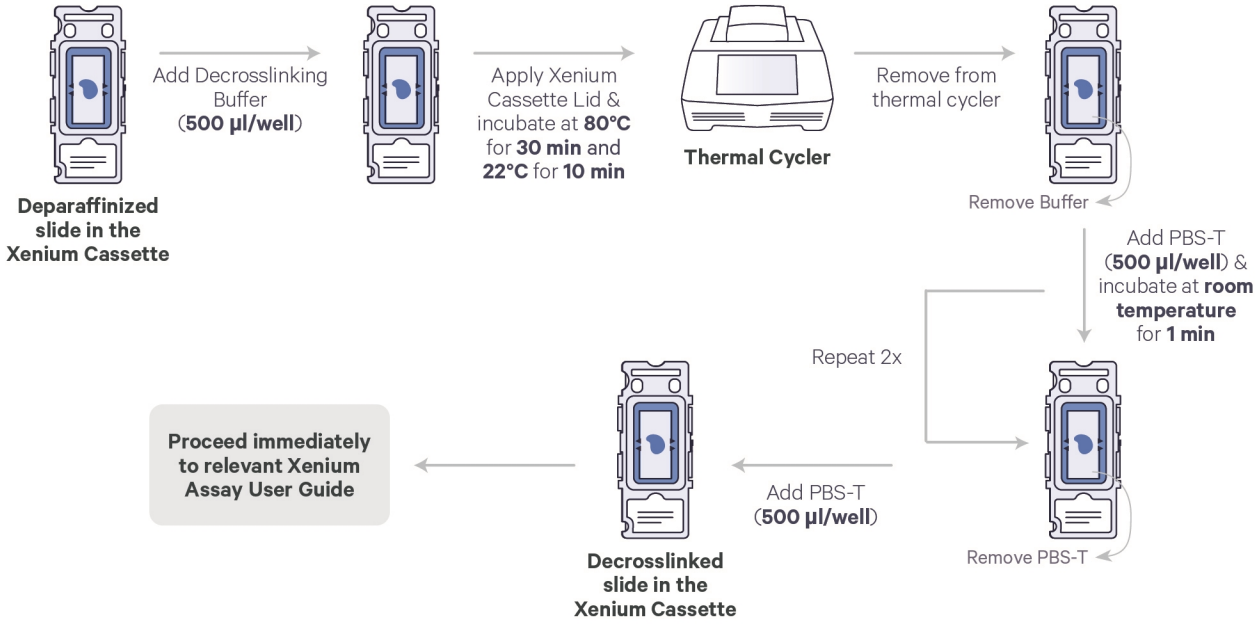
Protocol Overview

During Daparaffinization, the Xenium Slide incubation time at 60°C for:

- Xenium v1 is **2 h** (Deparffinization time is **~3 h**)
- Xenium Prime is **30 min** (Deparffinization time is **~1.5 h**)



Decrosslinking Sections ~1 h



Get Started - Deparaffinization & Decrosslinking

Each 10x Genomics reagent tube is good for two Xenium Slides.

Deparaffinization Items			10x PN	Preparation & Handling	Storage
Obtain					
☐		Xylene	-	-	Ambient
☐		Ethanol	-	Prepare Ethanol dilutions using Nuclease-free water.	Ambient
☐		Nuclease-free Water	-	-	Ambient
☐		10X PBS	-	-	Ambient
☐		Forceps	-	-	Ambient
☐		Slide Rack	-	-	Ambient
☐		Coplin jars/Staining dishes	-	-	Ambient
☐		Xenium Slides with FFPE tissue sections	3000941	Prepared according to Xenium In Situ for FFPE - Tissue Preparation Guide (CG000578).	Room temperature in a dessicator
	Xenium v1	Thermocycler Adaptor Xenium Cassette v1	3000954 3000951	See Tips & Best Practices	Ambient
	OR				
☐	Xenium Prime	Thermocycler Adaptor v2 Xenium Cassette Top v2 Xenium Cassette Bottom v2	3002207 3002205 3002223	See Tips & Best Practices	Ambient

The instructions apply to both Xenium v1 and Xenium Prime assays. The consumables are referred to generically without always citing the version suffix. Based on the assay choice, always ensure that either Xenium v1 or Xenium Prime specific reagents and consumables are used.

Decrosslinking Items			10x PN	Preparation & Handling	Storage
Equilibrate to room temperature					
☐		Perm Enzyme B	3000553	Maintain at room temperature. Pipette mix, centrifuge briefly. DO NOT vortex.	-20°C
☐		Xenium FFPE Tissue Enhancer*	2000798	Thaw in a thermomixer for 30 min at 37°C, 300 rpm with shaking. Cool to room temperature for 5 min. Vortex for 30 sec and centrifuge briefly. Alternatively, thaw in a water bath for 30 min at 37°C. Cool to room temperature for 5 min. Vortex for 30 sec and centrifuge briefly.**	-20°C
Obtain					
☐		Nuclease-free Water	-	-	Ambient
☐		1X PBS	-	Prepared at Step 1.1a.	Ambient
☐		10% Tween-20	-	-	Ambient
☐		Urea	-	-	Ambient
☐	Xenium v1	Thermocycler Adaptor Xenium Cassette Lid	3000954 3001046	See Tips & Best Practices	Ambient
		OR			
	Xenium Prime	Thermocycler Adaptor v2 Xenium Cassette Lid v2	3002207 3002206	See Tips & Best Practices	Ambient

**The reagent name may or may not include the prefix "Xenium"; Irrespective of the prefix, the indicated part number is associated with the reagent name.*

TIPS

**Pre-heat thermomixer or water bath to 37°C in advance of intended use.

1.1 Preparation - Buffers

For Deparaffinization:

Prepare all buffers fresh according to the tables below.

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 of this Demonstrated Protocol.

1X PBS				
Items		**Stock**	**Final**	**Total Amount (ml)**
☐	Nuclease-free water	-	-	9.0
☐	RNase free PBS, pH 7.4	10X	1X	1.0
☐	**Total**	**-**	**-**	**10.0**

b. Prepare eight total coplin jars for deparaffinization steps. Prepare Ethanol dilutions using Nuclease-free water.

For Deparaffinization		
Items		**Preparation & Handling**
☐	Xylene	Label two coplin jars as Xylene Jar 1 and 2. Fill to capacity with xylene in each.
☐	100% Ethanol	Label two coplin jars as 100% Ethanol Jar 1 and 2. Fill to capacity with 100% ethanol.
☐	96% Ethanol	Label two coplin jars as 96% Ethanol Jar 1 and 2. Fill to capacity with 96% ethanol.
☐	70% Ethanol	Label one coplin jar as 70% Ethanol Jar. Fill to capacity with 70% ethanol.
☐	Nuclease-free water	Label one coplin jar as Nuclease-free Water Jar. Fill to capacity with Nuclease-free 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. Prepare fresh reagents after every 20 slides or every week (whichever comes first).*

For Decrosslinking:

Prepare all buffers fresh according to the tables below.

c. Using 1X PBS from step 1.1a, 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 Demonstrated Protocol.

PBS-T				
Items		**Stock**	**Final**	**Total Amount (µl)**
☐	1X PBS	-	-	4,975.0
☐	Tween-20	10%	0.05%	25.0
☐	**Total**	**-**	**-**	**5,000.0**

d. Prepare Diluted Perm Enzyme B using 1X PBS prepared from step 1.1a. Add reagents in the order listed. Mix thoroughly with a 1-ml pipette set to 600 µl. Maintain at room temperature.

Diluted Perm Enzyme B				
Items		**Stock**	**Final**	**Total Amount (µl)**
☐	1X PBS	1X	-	998.0
☐	Perm Enzyme B *(Maintain at room temperature. Pipette mix, centrifuge briefly. DO NOT vortex).*	-	-	2.0
☐	**Total**	**-**	**-**	**1,000.0**

e. Prepare Decrosslinking Buffer using Diluted Perm Enzyme B prepared at step 1.1d. Add reagents in the order listed. Pipette mix thoroughly. Maintain at room temperature in the dark.

Decrosslinking Buffer					
Items		**Stock**	**Final**	**1 slide+10% (µl)**	**2 slides+10% (µl)**
☐	Xenium FFPE Tissue Enhancer *(Thaw in a thermomixer for 30 min at 37°C, 300 rpm with shaking. Cool to room temperature for 5 min. Vortex for 30 sec and centrifuge briefly. Alternatively, thaw in a water bath for 30 min at 37°C. Cool to room temperature for 5 min. Vortex for 30 sec and centrifuge briefly).*	-	-	508.8	1,017.5
☐	Urea	8 M	0.5 M	34.4	68.8
☐	Diluted Perm Enzyme B	-	-	6.9	13.8
☐	**Total**	**-**	**-**	**550.0**	**1,100.0**

1.2 Deparaffinization

Deparaffinization 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.

The instructions apply to both Xenium v1 and Xenium Prime assays. The consumables are referred to generically without always citing the version suffix. Based on the assay choice, always ensure that either Xenium v1 or Xenium Prime specific reagents and consumables are used.

a. Retrieve the slide with tissue sections from the desiccator after overnight drying.

Remove any marker annotations on slide using a lint-free laboratory wipe and 100% Ethanol.

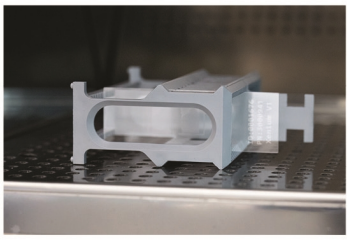
b. Place slide in a Section Dryer Oven and incubate uncovered at **60°C**: for

- Xenium v1 for **2 h**
- Xenium Prime for **30 min**

Keep the oven lid closed during incubation.

Incubation in a Section Dryer Oven

Place slide in a rack

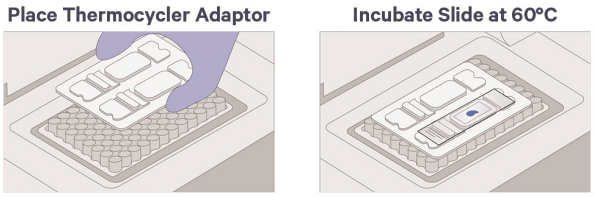


Place slide sideways and keep the oven door closed during incubation

Alternatively, place a Thermocycler Adaptor on a thermal cycler set at **60°C.** Place slide on the Thermocycler Adaptor with the tissue side facing up and incubate at **60°C**:

- Xenium v1 for **2 h**
- Xenium Prime for **30 min**

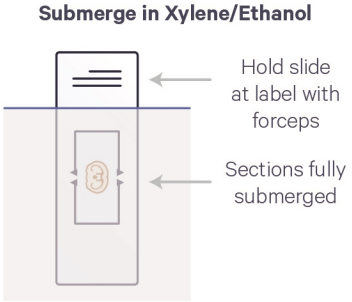
  DO NOT close the thermal cycler lid.



c. Remove from the oven or thermal cycler and allow the slide to cool down to **room temperature** for **7 min**.

Optional: photograph the slide against a black background during 7 min cool down at room temperature. This image can be used for comparison purposes to identify tissue detachment downstream in the workflow. Work quickly as this is not a safe stopping point. See Troubleshooting for more details.

d. Gently immerse slide in the Xylene Jar 1. Secure the jar cap to prevent xylene loss.



Hold slide at label with forceps for xylene immersion steps. When immersing slides in xylene, ensure that the tissue sections are completely submerged.

e. Incubate for **10 min**.

f. Gently immerse slide in the Xylene Jar 2 and incubate for **10 min**.

g. Gently immerse slide in the 100% Ethanol Jar 1 for **3 min**.

Hold slide at label with forceps for ethanol immersion steps. When immersing slides in ethanol, ensure that the tissue sections are completely submerged.

h. Gently immerse slide in the 100% Ethanol Jar 2 for **3 min**.

i. Gently immerse slide in the 96% Ethanol Jar 1 for **3 min**.

j. Gently immerse slide in the 96% Ethanol Jar 2 for **3 min**.

k. Gently immerse slide in the 70% Ethanol Jar for **3 min**.

l. Gently immerse slide in the Nuclease-free water Jar for **20 sec**.

1.3 Cassette Assembly

The instructions apply to both Xenium v1 and Xenium Prime assays. The consumables are referred to generically without always citing the version suffix. Based on the assay choice, always ensure that either Xenium v1 or Xenium Prime specific reagents and consumables are used.

a. Remove any remaining nuclease-free water from the slide using a lint-free laboratory wipe. Dry back of slide and front of slide outside of Sample Area completely without touching or damaging the tissue sections. Place the slide in the cassette.

TIPS

Refer to Cassette Assembly on page 41 for guidance on Xenium Cassette Assembly. Work quickly to avoid drying out of tissue sections.

b. Add **500 µl** 1X PBS.

Optional: photograph the slide against a black background. This image can be used for comparison purposes to identify tissue detachment downstream in the workflow. Work quickly as this is not a safe stopping point. See Troubleshooting on page 1for more details.

c. Proceed **immediately** to the next step.

Assembled Xenium Cassette

Xenium Cassette v1

Xenium Cassette v2

1.4 Decrosslinking

The instructions apply to both Xenium v1 and Xenium Prime assays. The consumables are referred to generically without always citing the version suffix. Based on the assay choice, always ensure that either Xenium v1 or Xenium Prime specific reagents and consumables are used.

Reagent addition and removal should be done carefully. Remove reagents along the side of the well. Do not touch tissue sections or introduce bubbles.

a. Place a Xenium Thermocycler Adaptor in the thermal cycler.

b. Prepare the thermal cycler with the following incubation protocol and start the program.

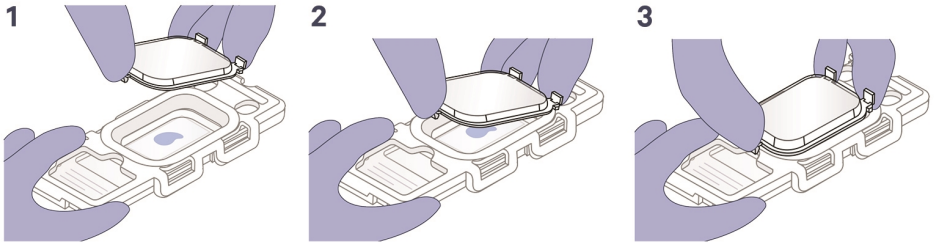
Lid Temperature	Reaction Volume	Run Time
80°C	100µL	-
Step	**Temperature**	**Time**
Hold	22°C	Hold
Decrosslinking	80°C	00:30:00
Re-equilibrate	22°C	00:10:00
Hold	22°C	Hold

c. Remove 1X PBS from step 1.3b.

d. Add **500 µl** Decrosslinking Buffer along the side of the well to uniformly cover the tissue sections, without introducing bubbles. Tap Xenium Cassette gently to ensure uniform coverage.

e. Apply a new Xenium Cassette Lid on the Xenium Cassette and place the cassette on the Thermocycler Adaptor at **22°C.** Close the thermal cycler lid.

Applying the Xenium Cassette Lid



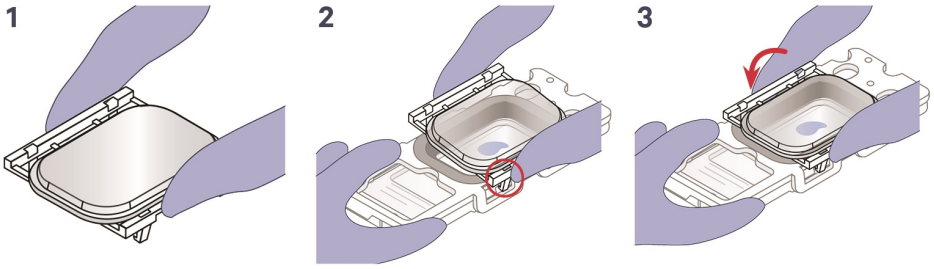
Hold the Xenium Cassette Lid with index and middle finger on two upper tabs and thumb on the lower clip.

Align lid with the surface of the Xenium Cassette. Hook the two upper clips into the two holes on the top of the cassette.

Push the lid down until the lower clip clicks into place. Inspect the lid to confirm placement.

OR

Applying the Xenium Cassette Lid v2



Hold the Xenium Cassette Lid v2 with thumb and index finger on the long sides.

On one side, align lid the lid feet to the notches on the cassette clip.. Hook the feet into the two side holes of the cassette.

Slowly push down on the other side of the lid until that side clicks into place. Inspect the lid to confirm placement.

f. Skip Hold step and initiate Decrosslinking.

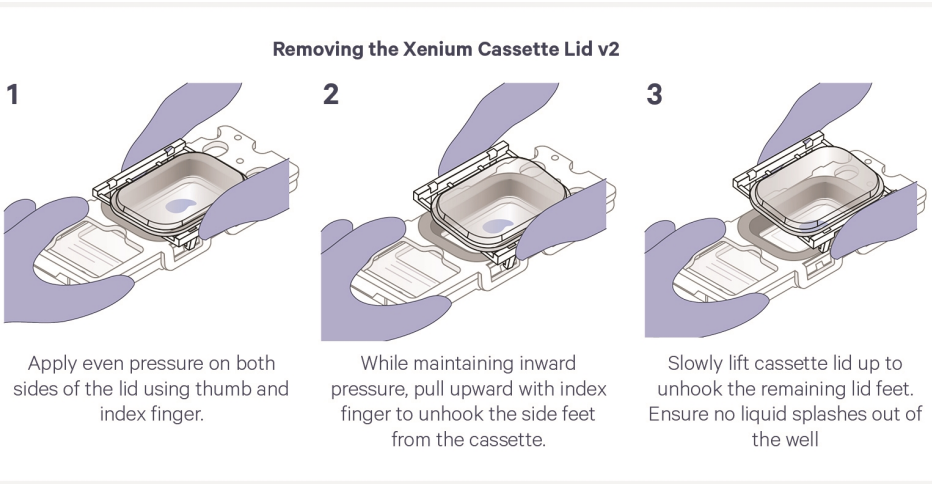
TIPS

Start thawing reagents for the relevant downstream workflow.

g. Remove Xenium Cassette from the thermal cycler and place on a flat, clean work surface.

h. Remove the Xenium Cassette Lid and using a pipette, remove all Decrosslinking Buffer from the well. **Discard** the old cassette lid.

OR



Three PBS-T Washes:

PBS-T washes DO NOT require sealing of the cassette with a lid.

i. **Wash 1:** Add **500 µl** PBS-T to the well. Incubate for **1 min** at **room temperature**. Remove all PBS-T.

j. **Wash 2:** Add **500 µl** PBS-T to the well. Incubate for **1 min** at **room temperature**. Remove all PBS-T.

k. **Wash 3:** Add **500 µl** PBS-T to the well. Incubate for **1 min** at **room temperature**. Remove all PBS-T.

l. Add **500 µl** PBS-T to the well.

Optional: photograph the slide against a black background. This image can be used for comparison purposes to identify tissue detachment downstream

in the workflow. Work quickly as this is not a safe stopping point. See Troubleshooting for more details.

m. Proceed directly to the relevant user guide:
This is not a safe stopping point.

- Xenium In Situ Gene Expression (CG000582)
- Xenium In Situ Gene Expression with Cell Segmentation Staining (CG000749)
- Xenium Prime In Situ Gene Expression with optional Cell Segmentation Staining (CG000760)

Troubleshooting

Tissue Detachment on Xenium Slides

Tissue sections may detach from Xenium slides during on-slide workflows. Tissue adhesion to the slide is impacted both by tissue processing and tissue quality.

Careful tissue preparation is critical for adhesion in on-slide workflows. Consult Xenium In Situ for FFPE - Tissue Preparation Guide (Document CG000579) for tissue QC and sectioning best practices. Below are some additional best practices for minimizing detachment during on-slide workflows:

- Do not pipette directly onto the tissue.
- Gently add and remove reagents from the well. Forceful addition or removal of reagents can agitate tissue and lead to detachment.
- Avoid touching in and around the Sample Area of the Xenium slide.
- Work quickly and carefully during reagent addition and removal.

In addition to following best practices, it is possible to monitor section adhesion on Xenium slides throughout the workflow. Taking a photograph of the slide at the beginning of the on-slide workflow and comparing with post-assay workflow images can help identify whether tissue shape has changed significantly, an indication of detachment. Steps when slide photos can be taken are noted in the protocol. These QC images can be compared with the DAPI overview scan as part of the Web Summary file to see whether tissue morphology has changed in the workflow.

If tissue detachment occurs, send pictures to support@10xgenomics.com for further assistance.

Cassette Assembly Failure

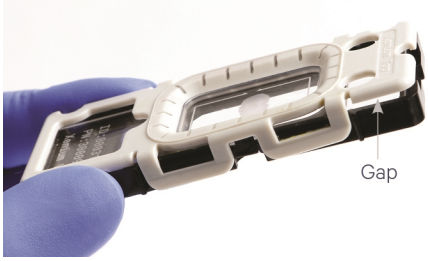
Incorrect assembly of the Xenium Cassette with a Xenium slide can negatively impact assay performance. Always dry the front and the back of the slide completely using a lint-free laboratory wipe while avoiding touching or damaging of the tissue sections. Inspect the slide carefully to ensure it is seated fully within the cassette before assembly. Additionally, inspect gasket before assembly to ensure it is not damaged or leaking.

Example scenarios that may indicate incorrect Xenium Cassette assembly are described below:

- If a gap appears between the two halves of the cassette after assembly.
- If the cassette does not click shut or appears domed after assembly.
- If the Xenium Thermocycler Adaptor is wet following removal from the thermal cycler, indicating reagent leakage from the cassette.

If the cassette is incorrectly assembled, disassemble and reassemble the cassette as instructed in Tips & Best Practices.

Incorrect cassette assembly as indicated by a gap between the two halves of the cassette

Correct cassette assembly	Incorrect cassette assembly
	

Document Revision Summary

Document Number	CG000580
Title	Xenium In Situ for FFPE – Deparaffinization & Decrosslinking Demonstrated Protocol
Revision	Rev E
Revision Date	August 2024
Specific Changes	• Updated PBS-T to PBS in 1.3 Cassette Assembly on page 41.
General Changes	Updated for general minor consistency of language and terms throughout.

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