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 In Situ Gene Expression protocols. Deparaffinized and decrosslinked tissue sections are inputs for the downstream Xenium In Situ Gene Expression - Probe Hybridization, Ligation & Amplification workflow.

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. After completing this Demonstrated Protocol (CG000580), proceed with the Xenium In Situ Gene Expression - Probe Hybridization, Ligation & Amplification User Guide (CG000582).

Contents

Xenium In Situ Gene Expression Reagent Kits

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	1	3000949
	Xenium Objective Wetting Consumable	1	2000749
●	Deionized Water	1	3001198
●	Xenium Sample Wash Buffer A	1	3001199
●	Xenium Sample Wash Buffer B	1	3001200
●	Xenium Probe Removal Buffer	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.

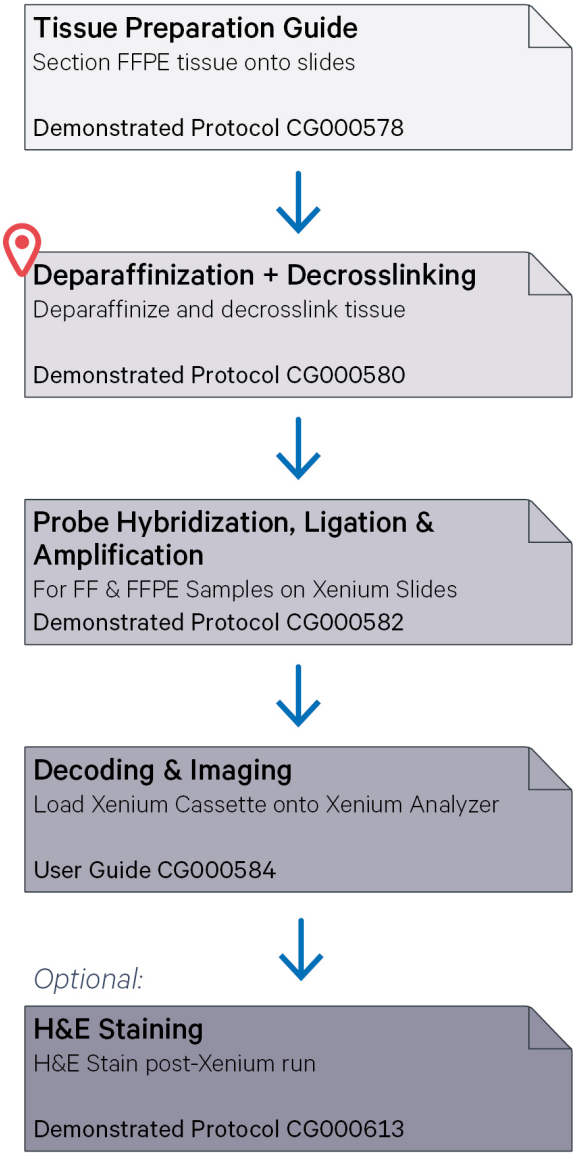
Recommended Thermal Cyclers

Supplier	Description	Part Number
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197

Thermal cycler requirements if reactions are performed on a slide:

- Temperature-controlled lid
- 96 deep-well block or 0.2 ml block configuration
- The thermal cycler must be able to accommodate the Xenium Thermocycler Adaptor:
  - Well depth: 4.5 mm
  - Distance between block and heated lid: 12 mm
  - Reaction block dimensions: 95.5 x 73 mm

Workflow Overview



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

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	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)	Thermo Fisher Scientific	28320
☐	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
☐	Blank Slides	Shandon ColorFrost Plus Slides 25 x 75 x 1 mm (Optional)	Thermal Fisher Scientific	6776214

For FFPE Tissue Sections: Deparaffinization & Decrosslinking			
	Fisherbrand Premier Plain Glass Microscope Slides (Optional)	Thermo Fisher Scientific	12-544-4
	Additional Materials		
☐	Waterbath or Thermomixer with 1.5 ml or 2 ml adapter		
☐	Thermal Cycler *(C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module, Bio-Rad, 1851197)*		
☐	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.

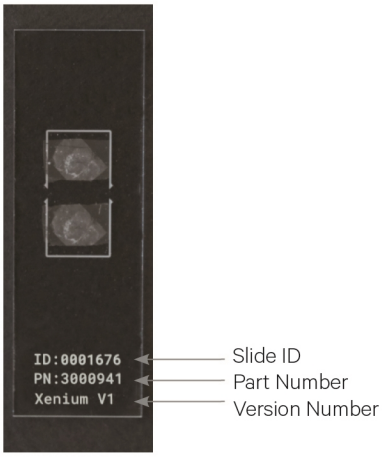
Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.

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.

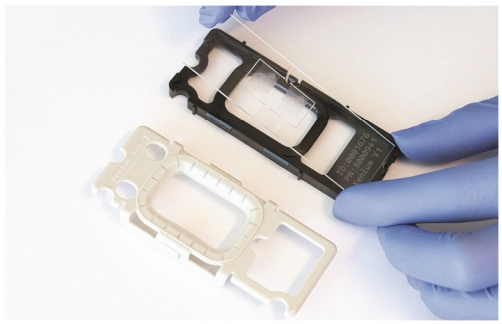
Xenium Slide with Tissue Sections



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.

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 dessicator and be used within one week.
- Store slides containing FFPE tissues at room temperature in a desiccator for up to four weeks (not in a sealed container).

Xenium Cassette

- 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.

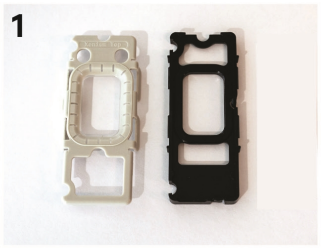
- The etched slide label is visible in the label window when properly assembled.
- Ensure that the Xenium Cassette and gasket are free of debris prior to assembly. If placing the top half of the cassette on a surface, ensure the gasket faces up so it does not collect debris.
- Visually inspect the gasket to ensure it is seated properly. If the gasket appears warped, the Xenium Cassette is still safe to use as long as the cassette can fully close.

Xenium Cassette Assembly

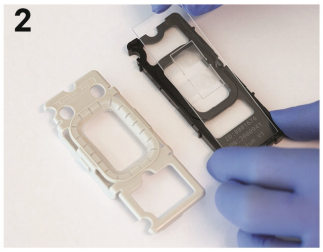
Ensure slide is completely dry before assembling into cassette.

Exercise caution when handling slide edges to prevent injury.

Place top and bottom halves of cassette on bench

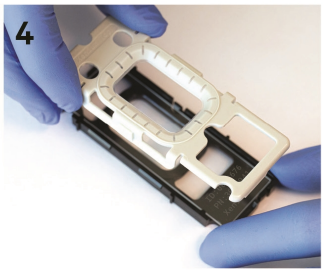


Place Xenium slide with tissue side facing upwards into bottom half of cassette; ensure label is toward bottom of cassette

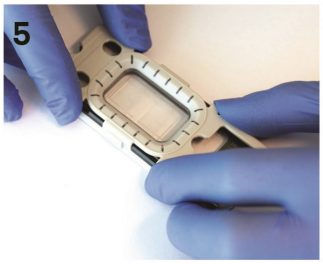


Press slide down into grooves of the bottom half of the cassette until it sits firmly in place

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



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

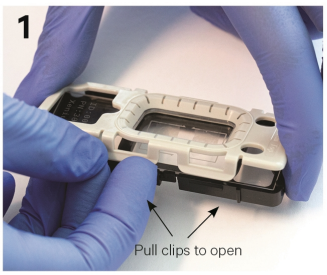


Slides in images are representative.

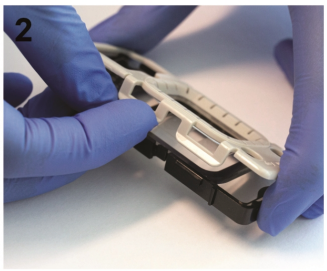
TIPS Once cassette is assembled, DO NOT remove slide until after Xenium Analyzer imaging and decoding for optional H&E staining step.

Xenium Cassette Removal

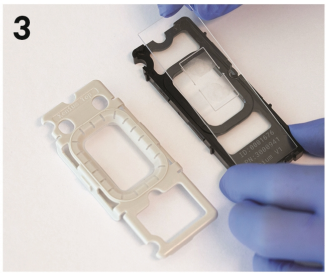
Pull inner clips from inner tabs to detach top and bottom halves of cassette



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.

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 Addition

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.

Reagent Removal

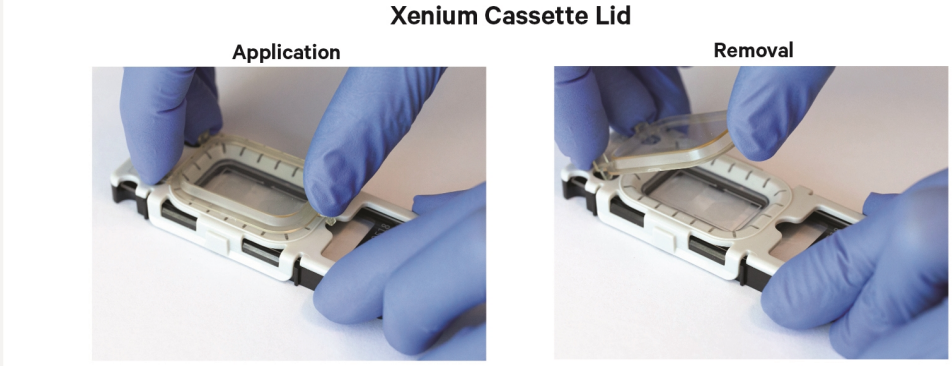
Xenium Cassette Lid Application & Removal

Application

- Place the Xenium Cassette flat on a clean work surface.
- Hold the Xenium Cassette Lid with index and middle finger on two upper tabs and thumb on the lower clip.
- Align the Xenium Cassette 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.

Removal

- Place the Xenium Cassette flat on a clean work surface.
- Push on the top of the two upper tabs with index and middle fingers.
- Use thumb to push in on the lower clip.
- While maintaining inward pressure, pull upward with thumb until the lower clip disengages.
- Ensure that no liquid splashes out of the well.



Note that Xenium Cassette Lids are a single use item and should be discarded after each use.

Slide Incubation Guidance

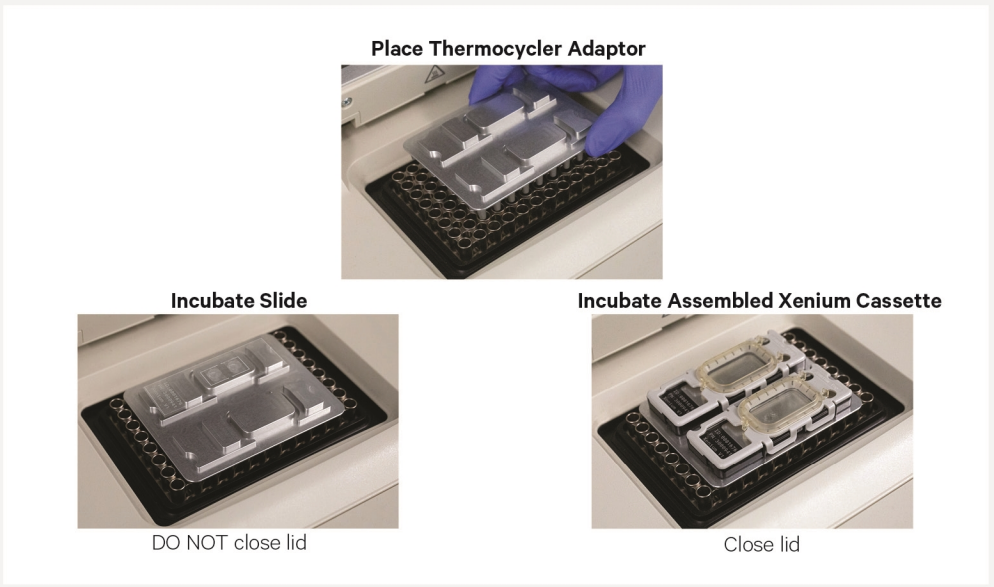
Incubation at a specified temperature

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.

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 prior to 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

- Xenium reagent kits are sufficient for two reactions, and for optimal Xenium Analyzer throughput, two slides should be run at the same time.

- It is possible to perform the Xenium In Situ Gene Expression 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 (2 cassettes), PN-1000566.
  - 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 Xenium Cassette Lid from the Xenium Cassette Kit (2 cassettes), PN-1000566 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 slide 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

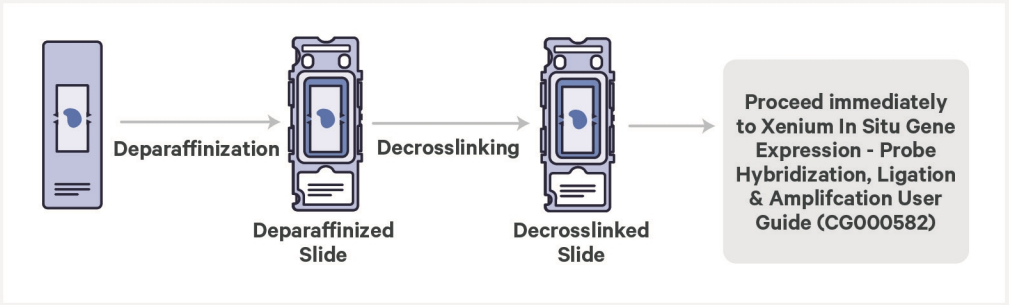
Steps		Timing	Stop & Store
Deparaffinization & Decrosslinking			
1.1	Buffer Preparation - Deparaffinization & Decrosslinking	30 min	
1.2	Deparaffinization *(includes 2 h baking at 60°C)*	3 h	
1.3	Cassette Assembly	10 min	
1.4	Decrosslinking	1 h	

Note there are no safe stopping points during this workflow.

1. Deparaffinization & Decrosslinking

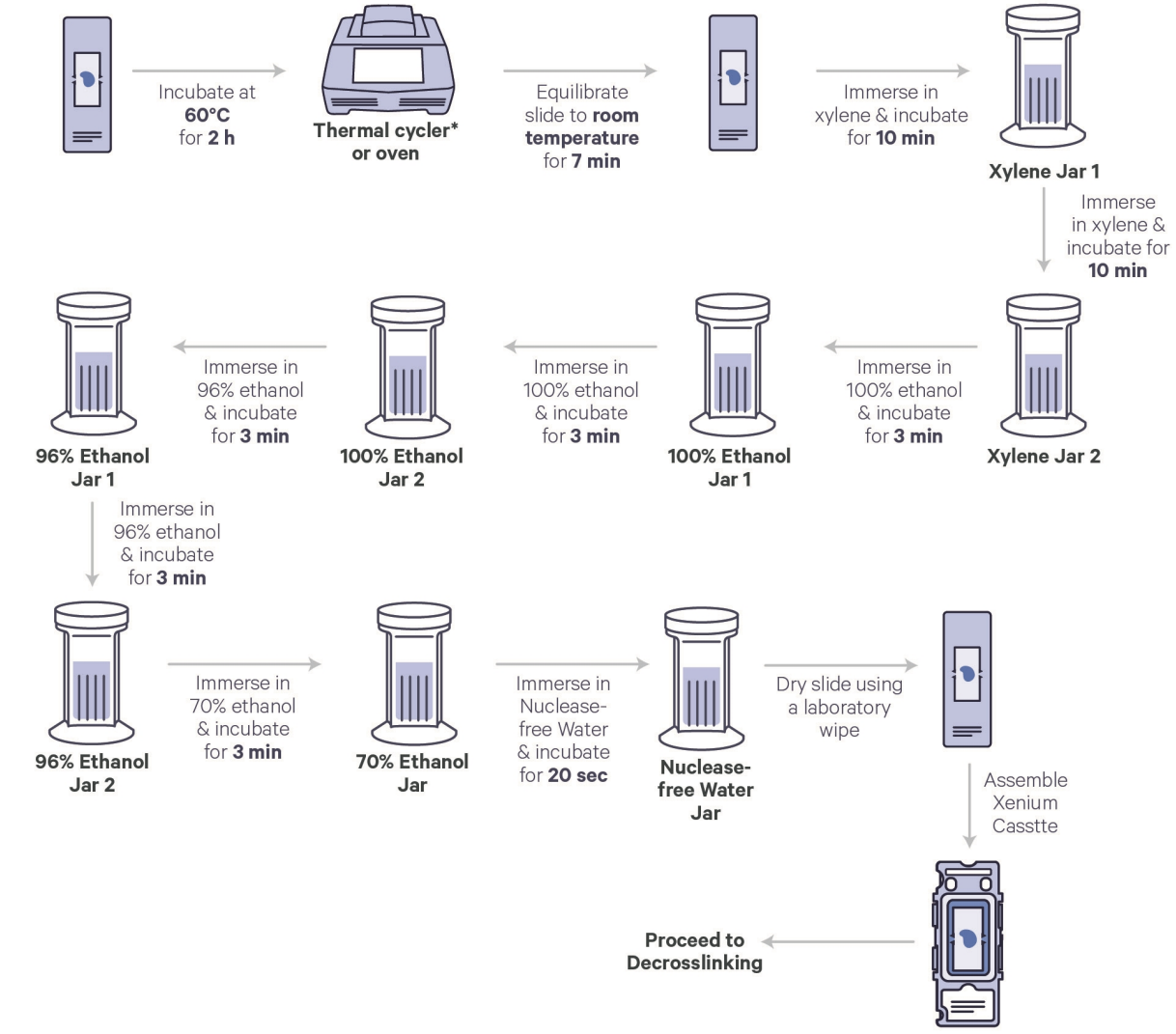
1.0 Overview

This chapter provides guidance on deparaffinization and decrosslinking of Xenium slides containing FFPE tissue sections that are dried overnight in a dessicator. After paraffin is removed from the tissue sections during the deparaffinization process, tissues are rehydrated, and subsequently decrosslinked to release the sequestered RNA from the tissue. Step 1 (Probe Hybridization) of Xenium In Situ Gene Expression - Probe Hybridization, Ligation & Amplification User Guide (CG000582) should be immediately performed following deparaffinization and decrosslinking.



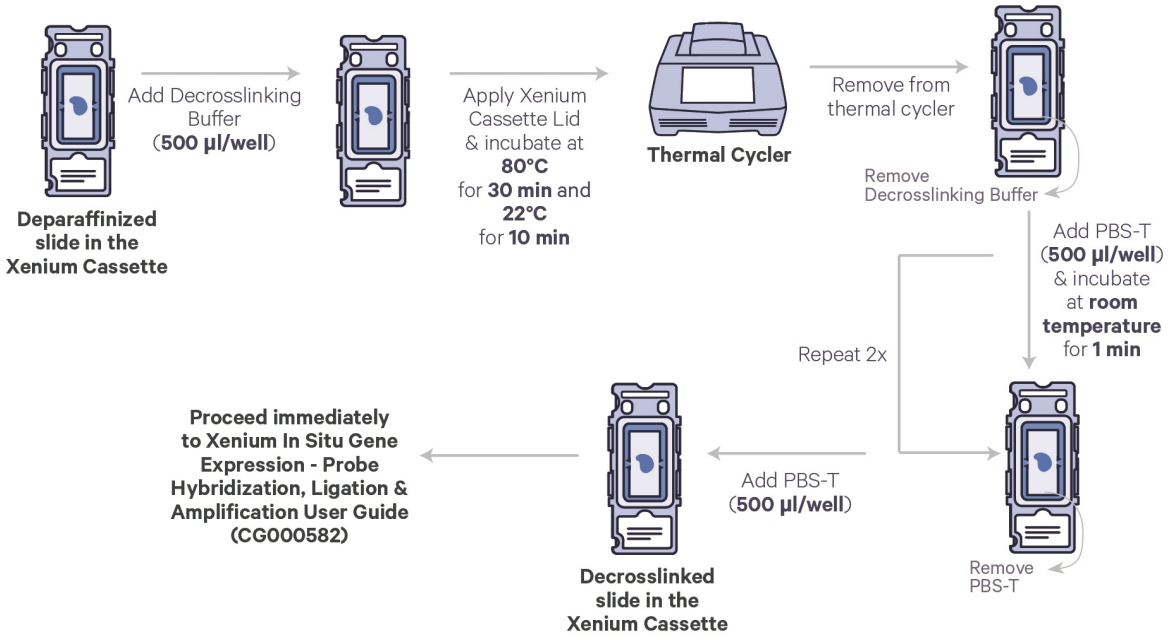
Protocol Overview

Deparaffinization ~3 h



Keep the thermal cycler lid open during incubation

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 (2 pack) with FFPE tissue sections	3000941	Prepared according to Xenium In Situ for FFPE - Tissue Preparation Guide (CG000578).	Room temperature in a dessicator

Decrosslinking Items		10x PN	Preparation & Handling	Storage
Equilibrate to room temperature				
☐	Perm Enzyme B	3000533	Thaw at room temperature. 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 waterbath 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

TIPS

*Pre-heat thermomixer or waterbath 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	10X	1X	1.0
☐	**Total**	**-**	**-**	**10.0**

b. Prepare eight total coplin jars for deparaffinization steps.

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 *(Thaw at room temperature. 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)**
☐	FFPE Tissue Enhancer Buffer *(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 waterbath 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.

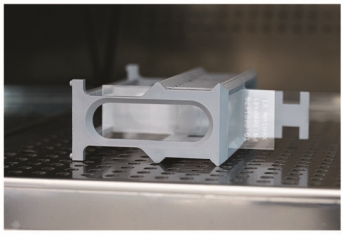
a. Retrieve the slide with tissue sections from the desiccator after overnight drying.

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 **2 h**. Keep the oven lid closed during incubation.

Incubation in a Section Dryer Oven

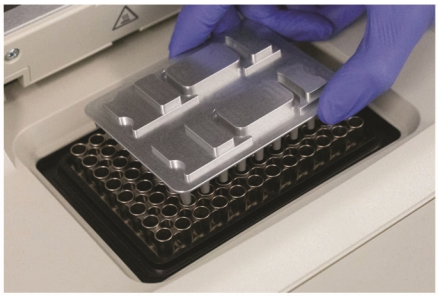
Place slide in a rack



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** for **2 h**. DO NOT close the thermal cycler lid.

Incubation in a Thermal Cycler



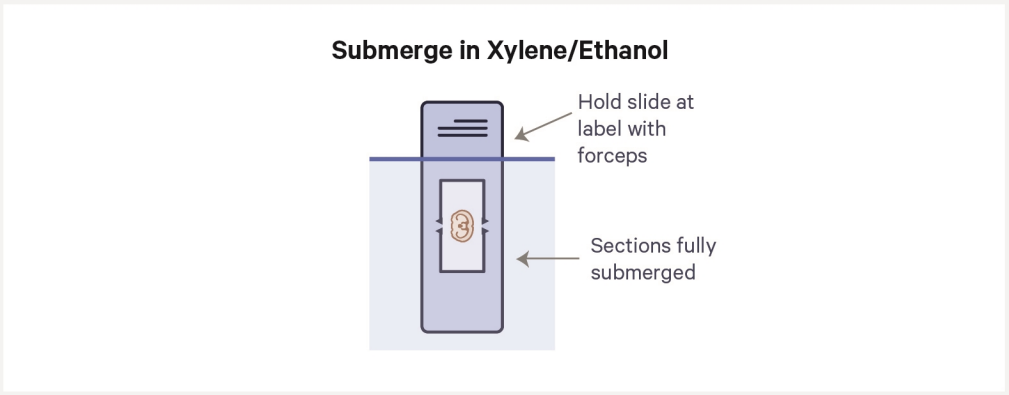
Place Thermocycler Adaptor

Incubate Slide for 2h at 60°C

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**.

TIPS

If processing two slides, leave second slide in water until the first cassette has been assembled and PBS-T has been added. See steps 1.3a-b of Cassette Assembly in the following section for more information.

1.3 Cassette Assembly

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

TIPS *Refer to Tips & Best Practices for guidance on Xenium Cassette Assembly.*

b. Add **500 µl** 1X PBS to the well.

c. Proceed **immediately** to Decrosslinking.

Assembled Xenium Cassette

1.4 Decrosslinking

Reagent addition and removal should be done carefully. Remove reagents along the side of the well without touching the tissue sections and without introducing 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.

f. Skip Hold step and initiate Decrosslinking.

TIPS

Start thawing reagents for Probe Hybridization during Decrosslinking incubation as indicated in the Xenium In Situ Gene Expression - Probe Hybridization, Ligation & Amplification User Guide (CG000582).

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 old Cassette Lids.

i. Add **500 µl** PBS-T to the well.

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

k. Carefully remove all PBS-T from the well using a pipette tip.

l. **Repeat** steps **i-k** two more times.

m. 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.

n. Proceed **immediately** to Xenium In Situ Gene Expression - Probe Hybridization, Ligation & Amplification User Guide (CG000582).

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

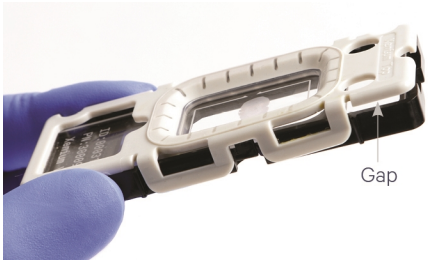
Incorrect assembly of the Xenium Cassette with a Xenium slide can negatively impact assay performance. Always dry the front and the back of the slide completely using a lint-free laboratory wipe while avoiding touching or damaging of the tissue sections. Inspect the slide carefully to ensure it is seated fully within the cassette before assembly.

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
	

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