

# Visium CytAssist Spatial Gene Expression for FFPE – Deparaffinization, H&E Staining, Imaging & Decrosslinking

## Introduction

The Visium CytAssist Spatial Gene Expression for FFPE assay is designed to analyze mRNA in tissue sections derived from formalin fixed & paraffin embedded (FFPE) tissue samples. The Visium CytAssist instrument requires a glass slide with intact tissue sections as input. This protocol outlines deparaffinization, Hematoxylin & Eosin (H&E) staining, imaging, and decrosslinking of tissue for use with 10x Genomics Visium CytAssist Spatial Gene Expression for FFPE assay. Deparaffinized, stained, and decrosslinked tissue sections are inputs for the downstream Visium Spatial Gene Expression for FFPE workflow.

## Additional Guidance

Consult the Visium CytAssist Spatial Gene Expression for FFPE - Tissue Preparation Guide (Document CG000518) for complete information on sectioning FFPE tissue blocks and placing sections on slides. Consult the Visium CytAssist Spatial Gene Expression for FFPE Imaging Guidelines (Document CG000521) to verify imaging settings prior to starting this Demonstrated Protocol. After completing this Demonstrated Protocol (CG000520), proceed with the Visium CytAssist Spatial Gene Expression for FFPE - User Guide (CG000495).

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## Reagent Kits

## Visium Spatial Gene Expression for FFPE Reagent Kits

See SDS for handling and disposal information

### Visium CytAssist Slide and Cassettes, 6.5 mm, 2 rxns PN-1000519

#### Visium CytAssist Slide and Cassettes, 6.5 mm

2 rxns

PN-1000519

Store at ambient temperature

	#	PN
Visium Cassette, 8 port	1	3000811
Visium Tissue Slide Cassette		
Visium CytAssist moveable gasket small (pre-assembled with translator)	2	3000814
Visium CytAssist moveable translator (preassembled with gasket)	2	3000816
Visium CytAssist moveable Cassette, frame	2	3000813
Visium CytAssist Slide Seals, 20 pack	1	2000284
Visium CytAssist Spatial Gene Expression Slide v2, 6.5 mm	1	2000549

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### Visium CytAssist Slide and Cassettes, 11 mm, 2 rxns PN-1000518

#### Visium CytAssist Slide and Cassettes, 11 mm

2 rxns

PN-1000518

Store at ambient temperature

	#	PN
Visium Cassette, 2 port	1	3000812
Visium Tissue Slide Cassette		
Visium CytAssist moveable gasket large	2	3000815
Visium CytAssist moveable Cassette, frame	2	3000813
Visium CytAssist Slide Seals, 20 pack	1	2000284
Visium CytAssist Spatial Gene Expression Slide v2, 11 mm	1	2000701

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## Reagent Kits

### Visium Spatial Gene Expression for FFPE Reagent Kits

Refer to SDS for handling and disposal information

#### Visium FFPE Reagent Kit v2 - Small PN-1000436

Visium FFPE Reagent Kit – Small		
PN-1000436		
Store at -20°C		
	#	PN
○ Amp Mix B	1	2000567
● Extension Enzyme	1	2000389
● Extension Buffer	1	2000409
● RNase Enzyme	1	3000593
● RNase Buffer B	1	2000551
● Tissue Removal Enzyme	1	3000387
● Tissue Removal Buffer B	1	2000543
● Tissue Removal Buffer Enhancer	1	2000557
● Decrosslinking Buffer*	1	2000566
● TS Primer Mix B	1	2000537
● Block and Stain Buffer	1	2000554



\*Only this reagent is used in this protocol.

Enough reagent is provided for processing two 6.5 mm slides or one 11 mm slide.

## 10x Genomics Accessories

Product	#	Part Number (Kit)	Part Number (Item)
Low Profile Plate Insert	2	1000499 (Visium CytAssist	3000823
10x Magnetic Separator	1	Reagent Accessory Kit)	230003 or 2000431

## Recommended Thermal Cyclers

Supplier	Description	Part Number
<b>Bio-Rad</b>	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
	PTC Tempo Deepwell Thermal Cycler*	12015392
<b>Eppendorf</b>	MasterCycler Pro (discontinued)	North America 950030010 International 6321 000.019
	MasterCycler X50s	North America 6311000010
<b>Thermo Fisher Scientific</b>	Veriti 96-Well Thermal Cycler (discontinued)	4375786
	VeritiPro Thermal Cycler, 96-well**	A48141
<b>Analytik Jena</b>	Biometra TAdvanced 96 SG with 96-well block (silver, 0.2 mL) and gradient function	846-x-070-241 (x=2 for 230 V; 4 for 115 V; 5 for 100 V)

\*For single cassette processing, place cassette on 10x Low Profile Thermocycler Adapter in the position farthest from the lid hinge to prevent lid locking errors.

\*\*Requires firmware 1.2.0 or later. Open lid slowly to avoid disturbing the cassette. After incubation, if the cassette becomes stuck to the lid, remove carefully. When working with tubes, VeritiPro requires blue MicroAmp 96-Well Tray/Retainer Set for Veriti Systems (PN-4381850), with top piece removed.



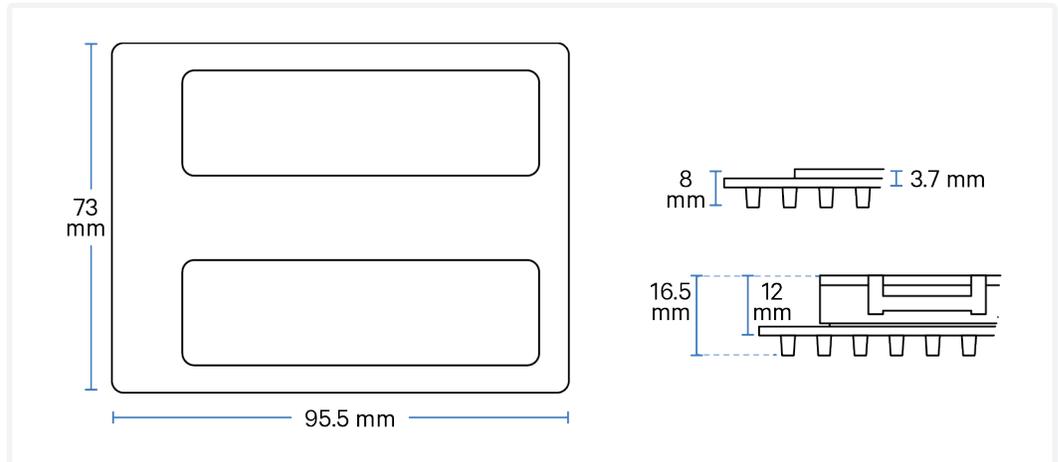
The following thermal cyclers should have their ramp rates adjusted for all steps:

- Eppendorf MasterCycler X50s: 3°C/sec heating and 2°C/sec cooling
- Analytik Jena Biometra TAdvanced: 2°C/sec heating and cooling

Thermal cycler must be able to accommodate the Low Profile Plate Insert (also referred to as the Low Profile Thermocycler Adapter):

- Well depth: 4.5 mm
- Distance between block and heated lid: 12 mm
- Reaction block dimensions 95.5 x 73 mm

The Low Profile Thermocycler Adapter may be difficult to remove from some thermal cyclers. If this is the case, turn off the thermal cycler and place a -20°C cooling pad on top of the Low Profile Thermocycler Adapter for a few minutes prior to attempting removal. Do not handle the Low Profile Thermocycler Adapter while it is hot.



## Workflow Overview

### 1 Experimental Planning

Before starting, see these documents.

#### Tissue Preparation Guide

Section tissue onto slides. Confirm compatibility of tissue on archived slides.

**Demonstrated Protocol**  
CG000518

#### Imaging Guidelines

Optimize imaging settings for Visium CytAssist Spatial Gene Expression for FFPE workflow.

**Technical Note**  
CG000521

### 2 Tissue & Sample Preparation

Detailed guidance for sample preparation.

#### Deparaffinization + H&E Staining + Decrosslinking

Deparaffinize, stain, image, and decrosslink tissue.

**Demonstrated Protocol**  
CG000520

### 3 Assay Workflow

Processed prepared tissue slides with the Visium CytAssist instrument.

#### Library Construction

Construct Visium Spatial Gene Expression - FFPE libraries.

**User Guide** CG000495

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

## Specific Reagents & Consumables

For each item, a number of vendor options are listed. Choose item based on availability and preference.

**Substituting materials may adversely affect system performance.**

Item	Alternatives/Options	Vendor	Part Number
Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
	Xylene, Histological Grade	Millipore Sigma	534056
Ethanol	Ethyl Alcohol, 200 Proof	Millipore Sigma	E7023
	Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only)	VWR	83813.360DP
Eosin	Eosin Y-solution, Alcoholic	Millipore Sigma	HT110116
	Eosin Y-solution, Alcoholic, with Phloxine	Millipore Sigma	HT110332
	Eosin Y Solution (Modified Alcoholic)	Abcam	ab246824
	Eosin Y with Phloxine 1% alcoholic solution	VWR	10143
Hematoxylin	Hematoxylin Solution, Mayer's	Millipore Sigma	MHS16
	Hematoxylin Solution According to Mayer	Millipore Sigma	51275
	Hematoxylin, Mayer's	Agilent	S330930-2
Bluing reagent	Bluing Reagent, Dako	Agilent	CS70230-2
	Thermo Scientific Shandon Bluing Reagent	Fisher Scientific	6769001
	Scott's Bluing Agent	Ricca Chemical Company	6697
Glycerol	Glycerol Solution	Millipore Sigma	49781
	Glycerol	Acros Organics	327255000
0.1 N HCl	Hydrochloric Acid Solution, 0.1 N <i>Or any equivalent HCl</i>	Fisher Chemical	SA54-1
Coplin jar/ staining dishes	Coplin Jar	VWR	100500-232
	Staining Dishes	VWR	25608-906
Section dryer oven	Epredia High Capacity Section Dryer <i>Or any equivalent product. Thermal cycler may also be used for section drying.</i>	Fisher Scientific	A84600051
Green Marker, <i>Optional, if annotating slide</i>	Sharpie Argyle Green Permanent Marker	Sharpie	1785396
Slide holders	Slide Holders, 24-place	VWR	25608-868
Coverslips	Fisherbrand Cover Glasses: Rectangles	Fisher Scientific	12-544-EP
	Cover Glasses, Rectangular	VWR	16004-322
Pipettes	Pipet-Lite Multi Pipette L8-200XLS+	Rainin	17013805
	Pipet-Lite LTS Pipette L-2XLS+	Rainin	17014393
	Pipet-Lite LTS Pipette L-10XLS+	Rainin	17014388
	Pipet-Lite LTS Pipette L-20XLS+	Rainin	17014392
	Pipet-Lite LTS Pipette L-100XLS+	Rainin	17014384
	Pipet-Lite LTS Pipette L-200XLS+	Rainin	17014391
	Pipet-Lite LTS Pipette L-1000XLS+	Rainin	17014382

## Specific Reagents & Consumables

For each item, a number of vendor options are listed. Choose item based on availability and preference.

**Substituting materials may adversely affect system performance.**

Item	Alternatives/Options	Vendor	Part Number
Wide Bore Pipette Tips	Tips RT LTS 200UL FLW	Rainin	30389241
	Tips RT LTS 1000UL FLW	Rainin	30389218
Pipette Tips	Tips LTS 200UL Filter RT-L200 FLR	Rainin	30389240
	Tips LTS 1ML Filter RT-L1000 FLR	Rainin	30389213
	Tips LTS 20UL Filter RT-L20 FLR	Rainin	30389226
<b>Additional Materials</b>			
1000-ml Beakers (7)		-	-
Ultrapure/Milli-Q Water, <i>from Milli-Q Integral Ultrapure Water System or equivalent</i>		-	-

## Tips & Best Practices

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Tips & Best Practices section includes additional guidance



Signifies critical step requiring accurate execution



Troubleshooting section includes additional guidance

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### General Reagent Handling

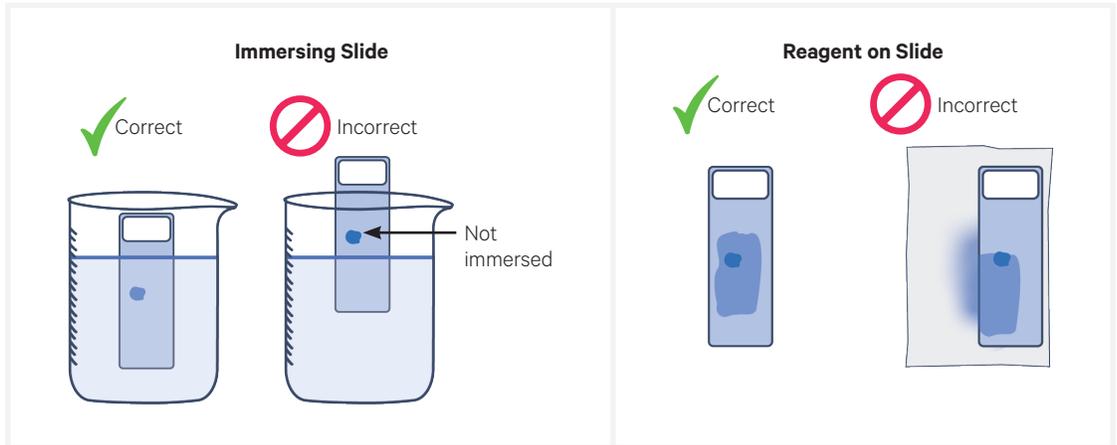
- Thoroughly mix reagents before use.
  - Use a pH meter to adjust pH as necessary during buffer preparation.
- 

### Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.
- 

### Slide Handling

- Always wear gloves when handling slides.
- DO NOT touch the tissue sections on the slide.
- Minimize exposure of the slides to sources of particles and fibers.
- When immersing slides in deparaffinization solutions and water, ensure that the tissue sections are completely submerged.
- Keep the slide flat on the bench when adding reagents to the tissue.
- Ensure that no absorbent surface is in contact with the reagents on the slide during incubation.
- Consider tracing the Tissue Slide Cassette gasket onto the back of the tissue slide to assist in alignment on the CytAssist instrument.
  - Markings may cause automatic tissue registration to fail, resulting in a need for manual registration.
  - If leaving a mark is desired, a green-colored marker (Sharpie, PN-1785396) has the least impact on the automatic tissue registration process.

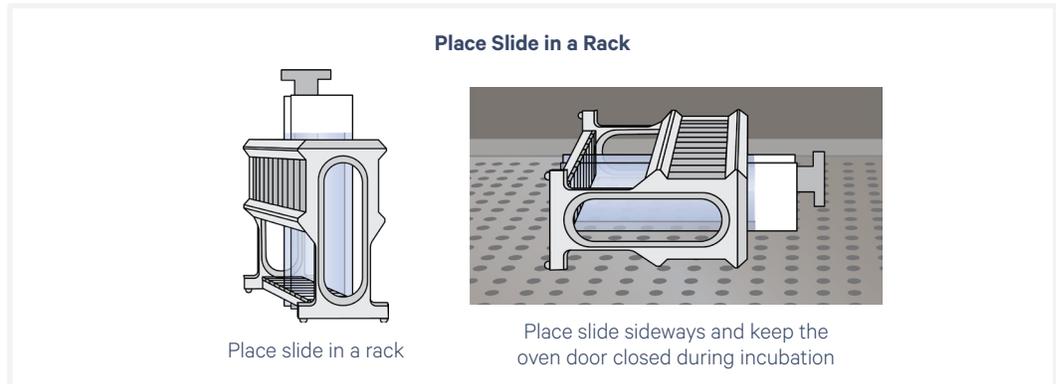


## Slide Incubation Guidance

### Incubation at a specified temperature

#### Incubation using a Section Dryer Oven:

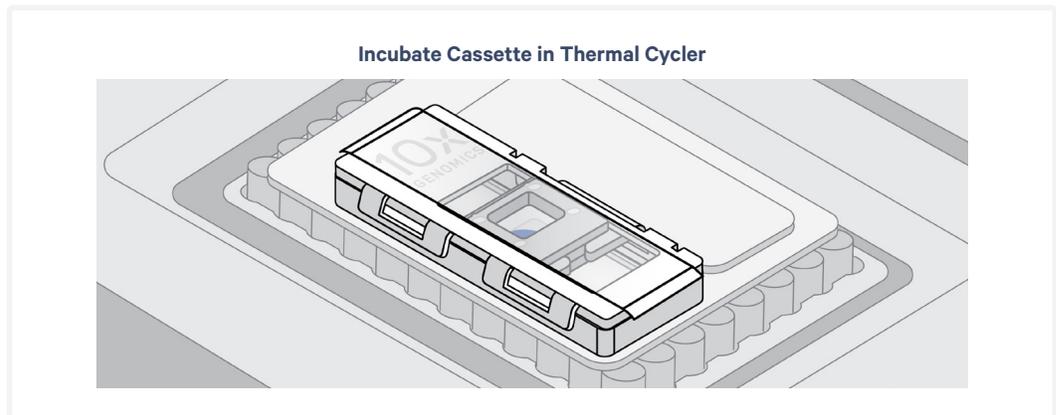
- Place the slides in a slide drying rack sideways to minimize paraffin wax entering neighboring tissue sections.



- Close the lid when incubating the slide in the oven.

#### Incubation using a Thermal Cycler:

- Position a Low Profile Plate Insert (also referred to as Low Profile Thermocycler Adapter) on a thermal cycler that is set at the incubation temperature. Move Low Profile Thermocycler Adapter back and forth to ensure that it is seated properly.
- Ensure that the Low Profile Thermocycler Adapter is in contact with the thermal cycler surface uniformly.
- When incubating a slide, position the slide on the Low Profile Thermocycler Adapter with the tissue surface facing up.
- Ensure that the entire bottom surface of the slide is in contact with Low Profile Thermocycler Adapter.
- When incubating a slide encased in a cassette, place the assembled unit on the Low Profile Thermocycler Adapter with the wells facing up. Cassettes should always be sealed when in the Low Profile Thermocycler Adapter.
- Allow Low Profile Thermocycler Adapter to cool before removing it from the



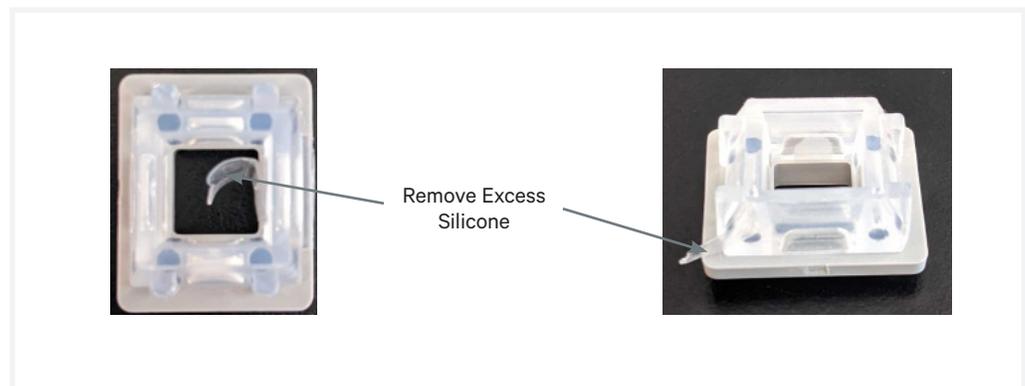
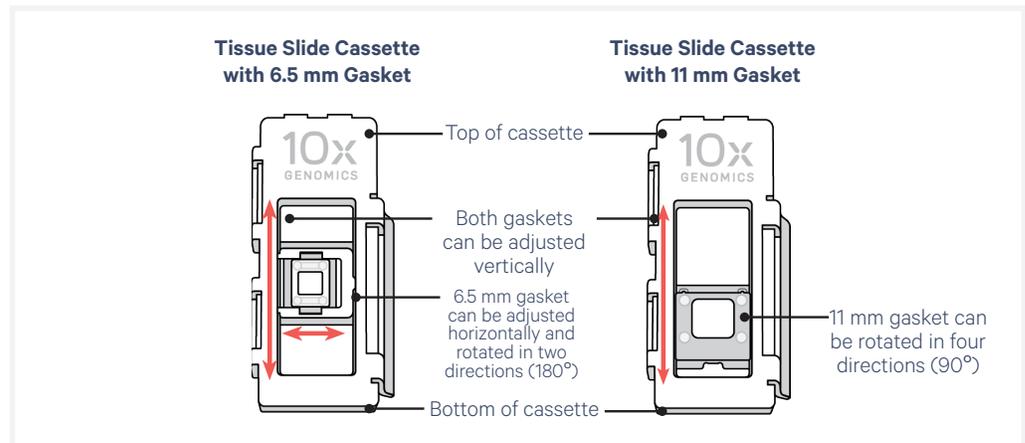
thermal cycler.

## Incubation at room temperature

- Place the Visium CytAssist Tissue Slide Cassette on a flat, clean, nonabsorbent work surface.

## Visium CytAssist Tissue Slide Cassette

- The Visium CytAssist Tissue Slide Cassette encases the slide and creates leak-proof wells for adding reagents on blank slides with tissue.
- The cassette is a single-use item.
- Gaskets are adjusted by the user to ensure that the tissue section or area of interest is encased in a well.
- See [Visium CytAssist Tissue Slide Cassette Assembly & Removal](#) instructions for details.
- Prior to use, inspect the moveable gasket to ensure that the gasket perimeter and corners are free of excess silicone prior to assembly.
- Excess silicone should be safely removed with forceps or a pipette tip prior to assembly.
- Assembly should occur against a white background for easy tissue visualization during alignment.
- Practice assembly with a blank slide (75 x 25 x 1 mm).



## Visium CytAssist Tissue Slide Cassette Assembly



Wear fresh gloves while assembling Tissue Slide Cassette

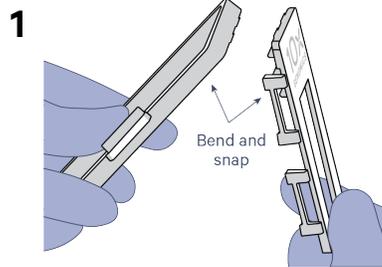


DO NOT close upper and lower halves of cassette before detaching hinges.

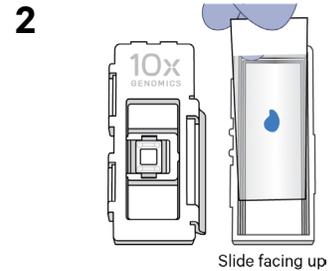


Exercise caution when handling slide edges to prevent injury.

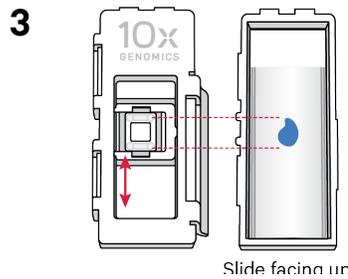
If applicable, break cassette into two halves by bending each half at the hinge until they snap apart



Place tissue slide into lower half of cassette with tissue facing up

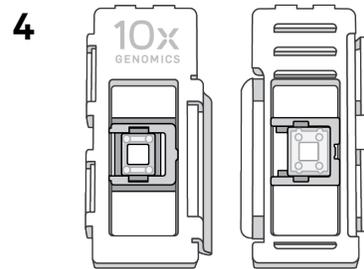


The 6.5 mm gasket can be adjusted horizontally and rotated in two directions (180°) while 11 mm gasket can be rotated in four directions (90°). Determine the appropriate configuration that allows the gasket to encompass the tissue area of interest.

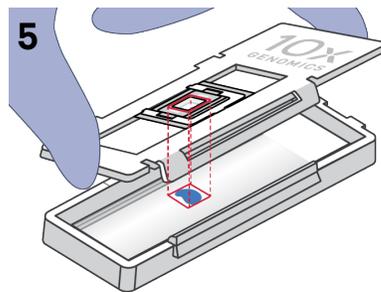


Gently place top half of cassette over bottom half. DO NOT assemble together until Step 7.

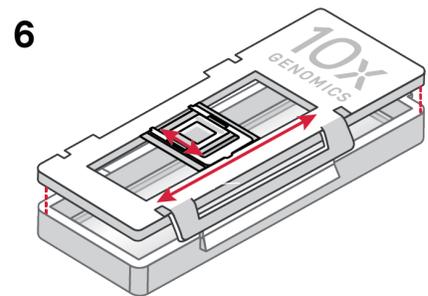
Securely combine gasket with top half of cassette until the gasket snaps into place.



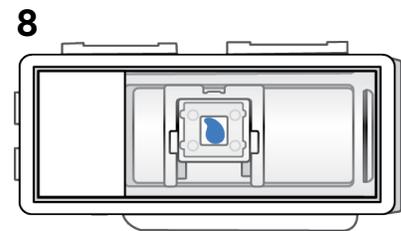
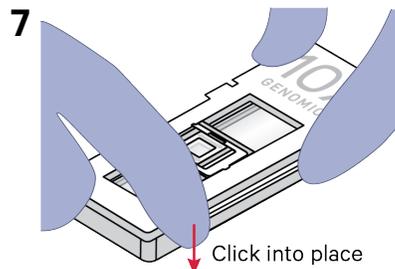
Adjust gasket such that gasket is over the tissue area of interest. The 6.5 mm gasket can be adjusted horizontally as well as vertically.



Apply even pressure on top of cassette until it clicks shut. Verify that clip is secured over hinges.



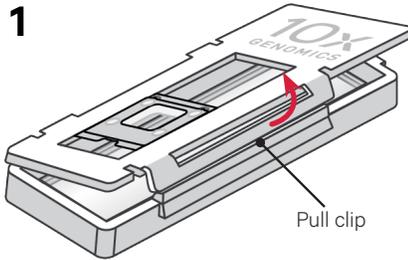
Turn cassette over and verify tissue area of interest is within gasket. DO NOT move gasket once cassette is closed. If necessary, open cassette and recenter gasket.



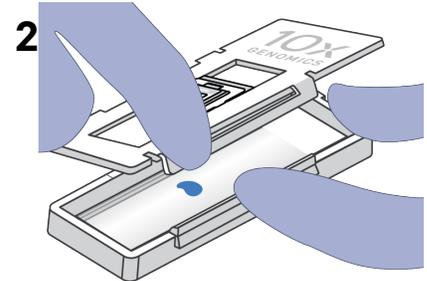
## Visium CytAssist Tissue Slide Cassette Removal

Removal instructions are the same for both 6.5 and 11 mm cassettes.

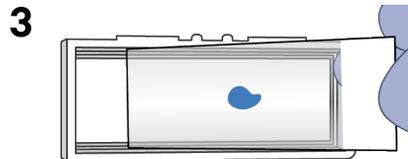
Pull clip up to detach upper and lower halves of cassette



Open cassette by continuing to lift clip upward. If slide sticks to gasket, continue to apply even upward pressure to separate slide from gasket

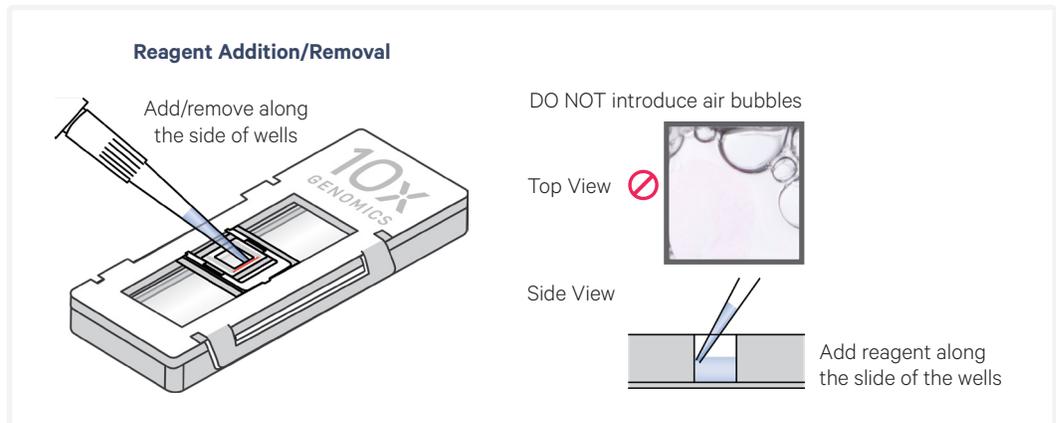


Hold slide by the label and lift slide out from lower half



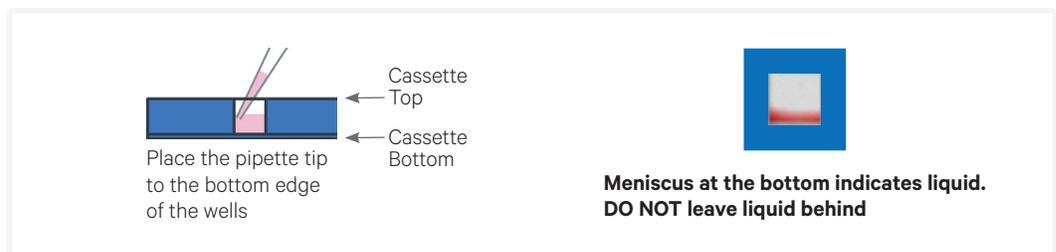
## Reagent Addition to Wells

- Place the assembled slide in the Visium CytAssist Tissue Slide Cassette flat on a clean work surface.
- Dispense reagents along the side of the wells without touching the tissue sections and without introducing bubbles.
- Always cover the tissue section completely when adding reagents to the well. A gentle tap may help spread the reagent more evenly.



## Reagent Removal from Wells

- Slightly tilt the cassette while removing the reagent.
- Place the pipette tip to the bottom edge of the wells.
- Remove reagents along the side of the wells without touching the tissue sections and without introducing bubbles.
- Remove all liquid from the wells in each step. To ensure complete removal, check the bottom of the well by tilting the cassette slightly. A meniscus at the bottom of the well will indicate the presence of liquid in the well. Repeat removal steps until no reagent remains.



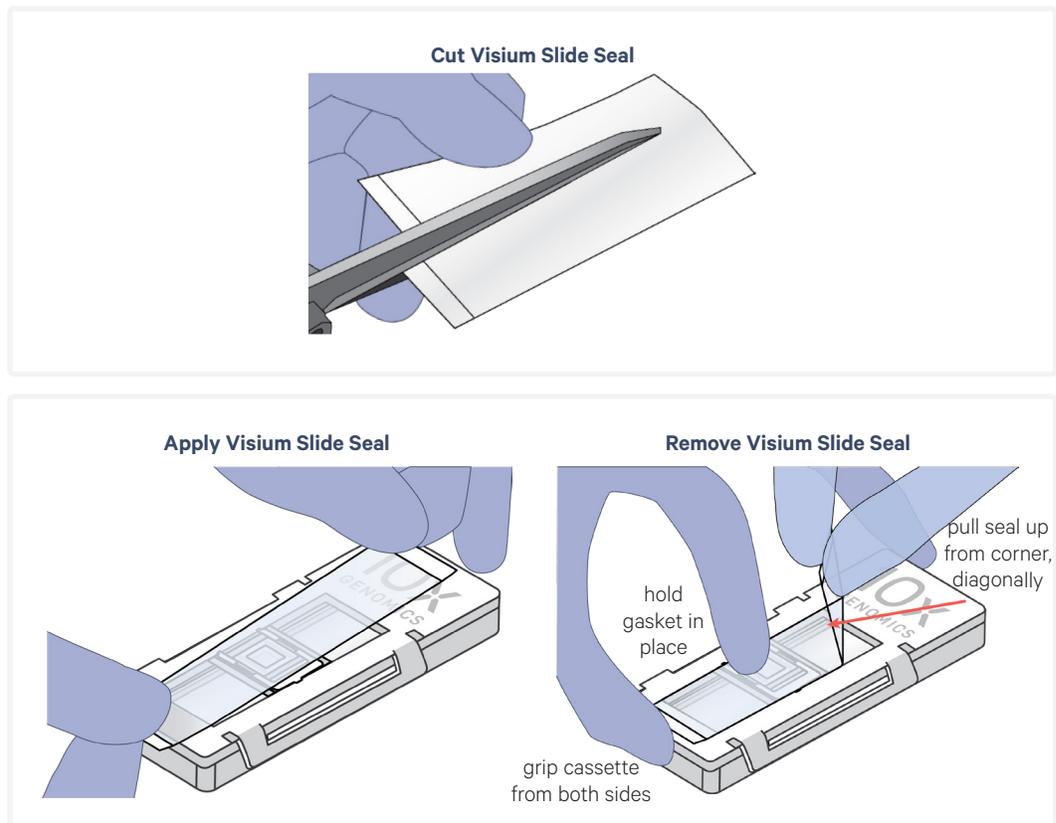
## Visium CytAssist Slide Seal Application & Removal

### Application

- If applying a Visium Slide Seal to a Tissue Slide Cassette, the seal must be cut in half lengthwise. Cut the seal as shown in the image below. Ensure scissors are cleaned with a RNase decontamination solution.
- Place the CytAssist Tissue Slide Cassette flat on a clean work surface.
- Remove the back of the adhesive Visium Slide Seal.
- Align the Visium Slide Seal with the surface of the cassette and apply while firmly holding the cassette with one hand.
- Press on the Visium Slide Seal to ensure uniform adhesion.

### Removal

- Place the CytAssist Tissue Slide Cassette flat on a clean work surface.
- Carefully pull Visium Slide Seal up and over from the edge while firmly holding the cassette.
- Ensure that no liquid splashes out of the wells.



## Visium CytAssist Tested Slides

The following slides have been tested for use with the Visium CytAssist instrument.

Item	Length (mm)	Width (mm)	Thickness (mm)	Ground Corners
Epredia Shandon ColorFrost Plus	75.0	25.0	1.0	No
Fisherbrand SuperFrost Plus	75.0	25.0	1.0	Available as either
Sigma-Aldrich Poly Prep Slides	75.0	25.0	1.0	No
VWR SuperFrost Plus Micro Slide, Premium	75.0	25.0	1.0	No

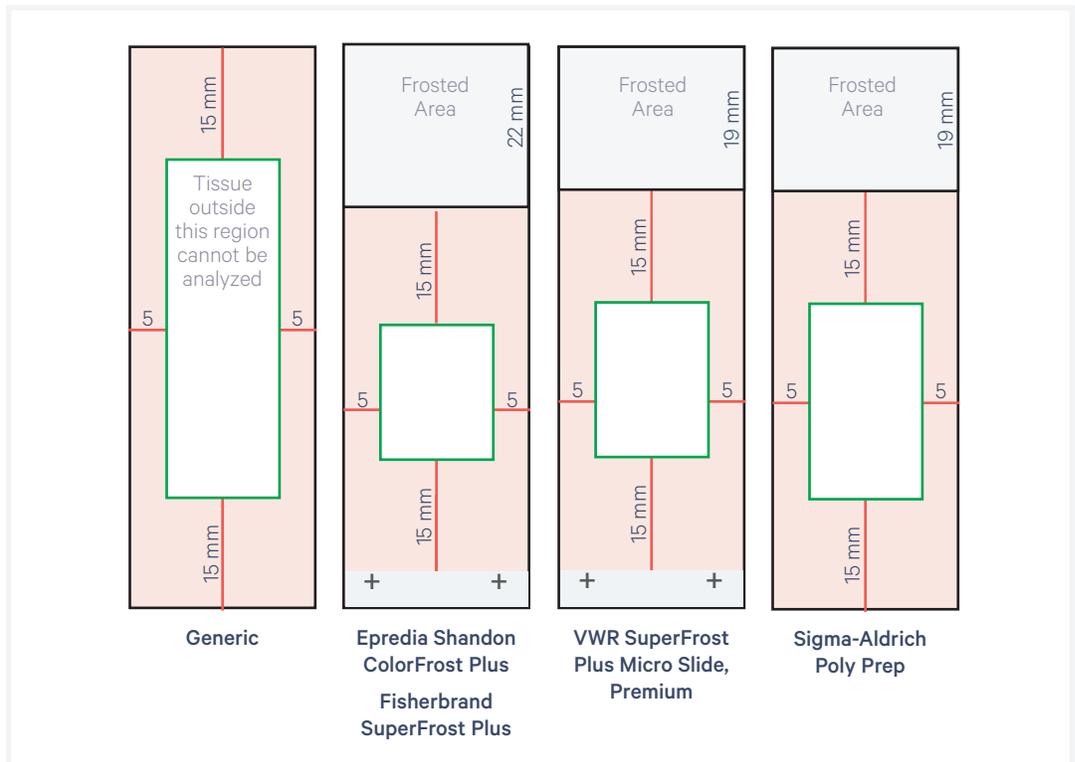
If unsure of slide part number, see the "generic" slide diagram below for general guidance (images not to scale). Diagrams for verifying that tissue sections are placed in the allowable area can also be found in the Visium CytAssist Quick Reference Cards - Accessory Kit (Document CG000548). The diagrams demonstrate allowable areas that are far enough away from frosted sections to not interfere with gasket closure during the CytAssist assay. Frosted sections include the opaque area of the slide as well as any etching or writing on the slide.



While slides are specified as being 25 mm x 75 mm, manufacturing tolerances may lead to dimensions that are too small or large to be compatible with 10x Genomics products. Tissue slide dimensions must be within 24.8 mm - 25.3 mm in width and 74.4 mm - 76.2 mm in length to fit the Visium CytAssist Tissue Slide Cassette.

Minimum slide dimensions: 24.8 x 74.4 mm

Maximum slide dimensions: 25.3 x 76.2 mm



# 1. Deparaffinization & H&E Staining

## 1.0 Overview

This chapter provides guidance on deparaffinization and H&E staining of tissue slides containing FFPE tissue sections that are dried overnight in a desiccator. Ensure that microscope settings have been verified and imaging programs have been created prior to starting this program. Consult the Visium Spatial Gene Expression for FFPE Imaging Guidelines Technical Note (CG000521) for more information.

If processing an archived slide that has gone through hardset coverslip removal and deparaffinization, proceed directly to Step 3: Decrosslinking.



## 1.1 Preparation - Buffers

### For Deparaffinization

Prepare fresh weekly, process two slides per jar. Alternatively, use a slide staining dish. Adjust volumes of deparaffinization solutions and water accordingly.

Items	Preparation & Handling
<input type="checkbox"/> Xylene	Label two coplin jars as Xylene Jar 1 and 2. Dispense 30 ml xylene in each.
<input type="checkbox"/> 100% Ethanol	Label two coplin jars as 100% Ethanol Jar 1 and 2. Dispense 30 ml 100% ethanol in each. <i>Alternatively, use a 50-ml centrifuge tube or a beaker.</i>
<input type="checkbox"/> 96% Ethanol	Label two coplin jars as 96% Ethanol Jar 1 and 2. Dispense 30 ml 96% ethanol in each. <i>Alternatively, use a 50-ml centrifuge tube or a beaker.</i>
<input type="checkbox"/> 70% Ethanol	Label one coplin jar as 70% Ethanol Jar. Dispense 30 ml 70% ethanol. <i>Alternatively, use a 50-ml centrifuge tube or a beaker.</i>
<input type="checkbox"/> Milli-Q or UltraPure Water	Label one coplin jar as Water Beaker 1. Dispense 30 ml water. <i>Alternatively, use a 50-ml centrifuge tube or a beaker.</i>



*Use xylene-resistant dishes for immersion in xylene.  
Use xylene-resistant gloves or forceps for deparaffinization.  
Prepare fresh reagents every week.*

For H&E Staining	
Items	Preparation & Handling
<input type="checkbox"/> Milli-Q or UltraPure Water	Label six 1000-ml beakers as Water Beakers 2-7. Dispense 800 ml of water into each beaker. Dispensed volumes in each beaker can be used for two slides. <i>Alternatively, use 50-ml centrifuge tubes instead of beakers.</i>
<input type="checkbox"/> Alcoholic Eosin	30 ml in a 50-ml conical tube for each tissue slide.

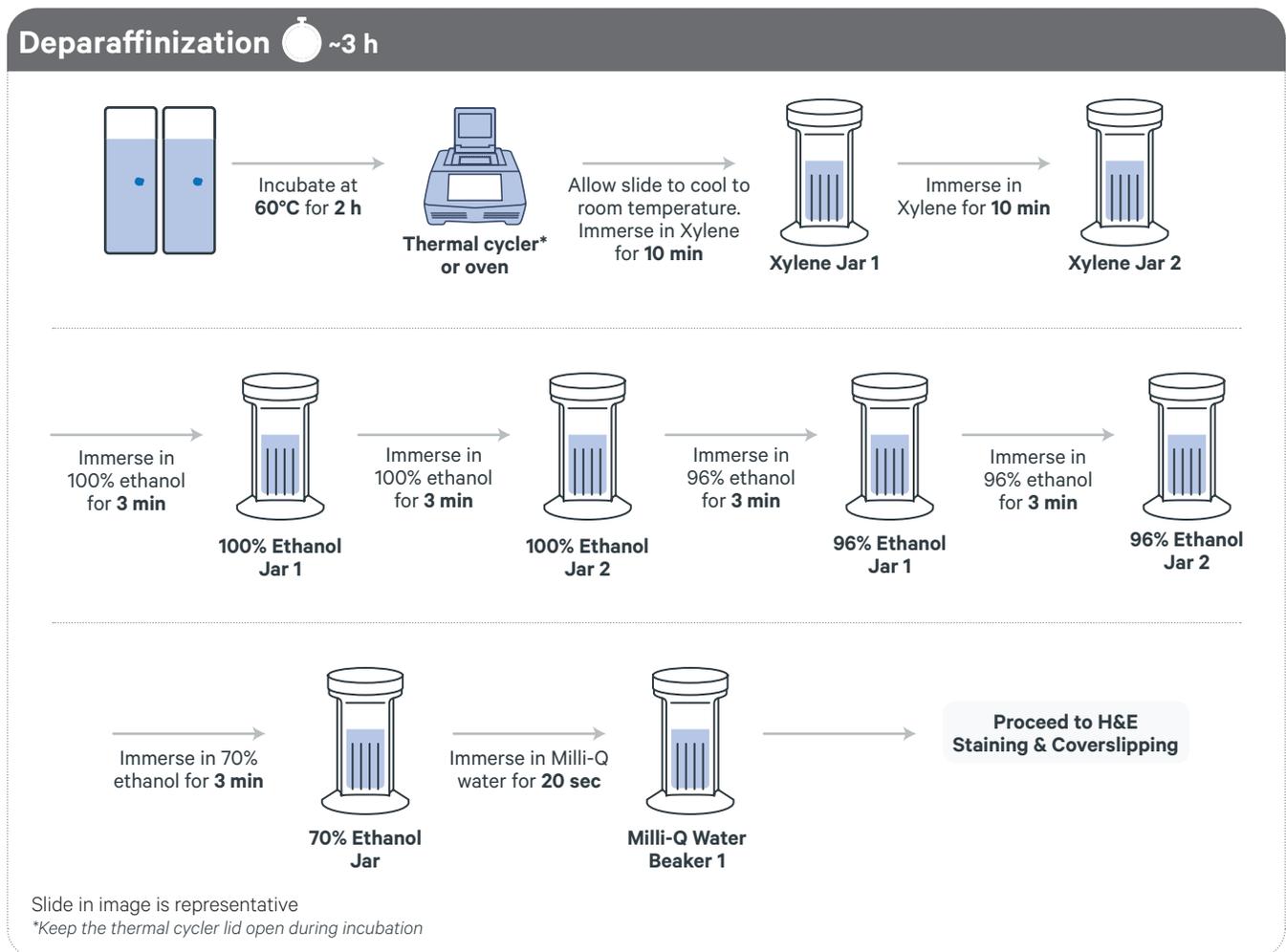
  

For Coverslipping	
Items	Preparation & Handling
<input type="checkbox"/> Mounting Medium	The dilution below is not necessary if stock glycerol is already at 85%. Invert to mix. Briefly centrifuge to remove bubbles.

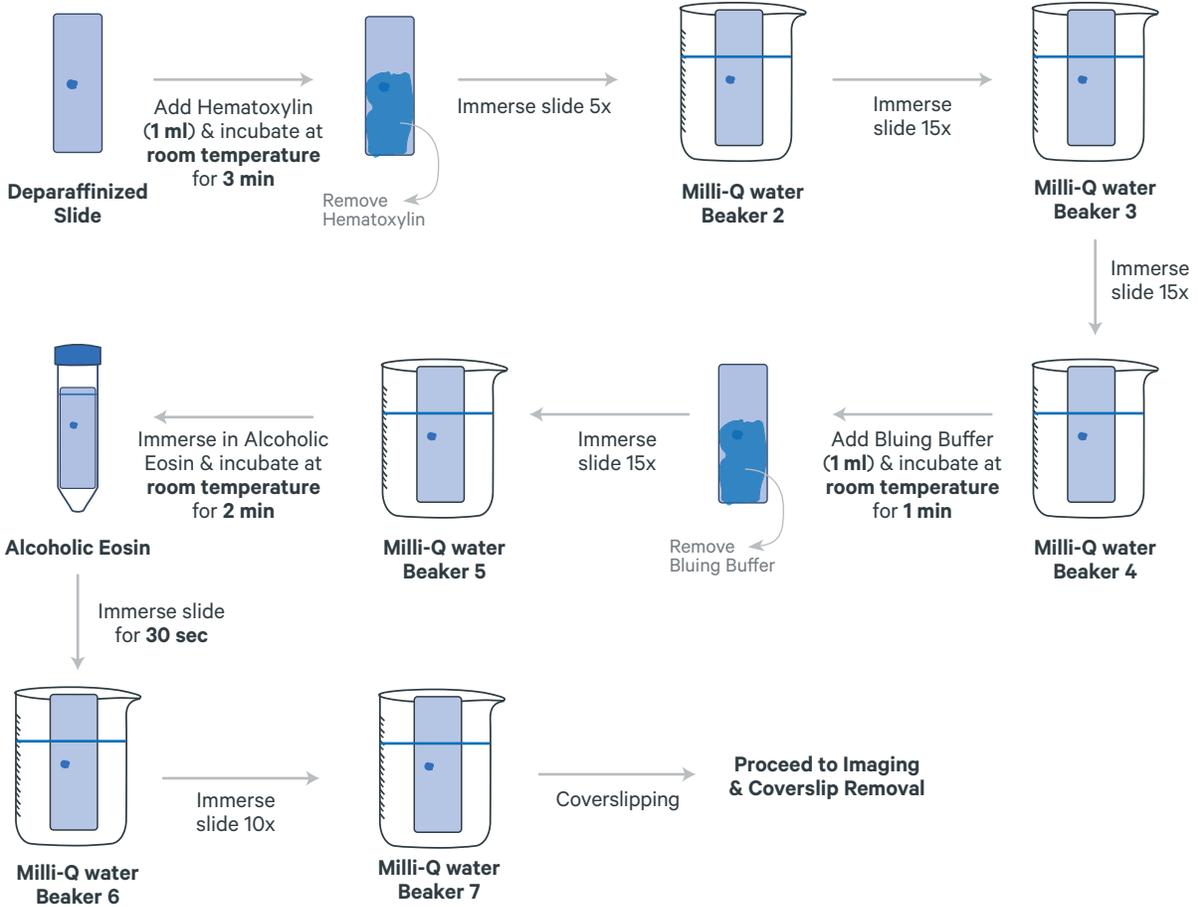
Mounting Medium	Stock	Final	1X (µl)	2X+ 15% (µl)
Glycerol	100%	85%	127.5	293.3
Nuclease-free Water	100%	15%	22.5	51.7
<b>Total</b>	-	-	<b>150.0</b>	<b>345.0</b>

## Protocol Overview



## Protocol Overview

### H&E Staining & Coverslipping ~15 min

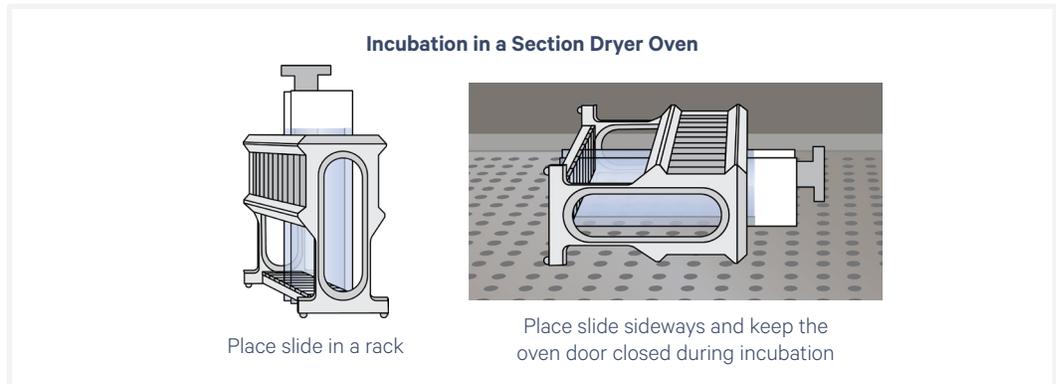


\*Wipe excess liquid from the back of the slide after each immersion series

## 1.2 Deparaffinization

Deparaffinization steps should be performed in a fume hood due to the hazardous nature of xylene.

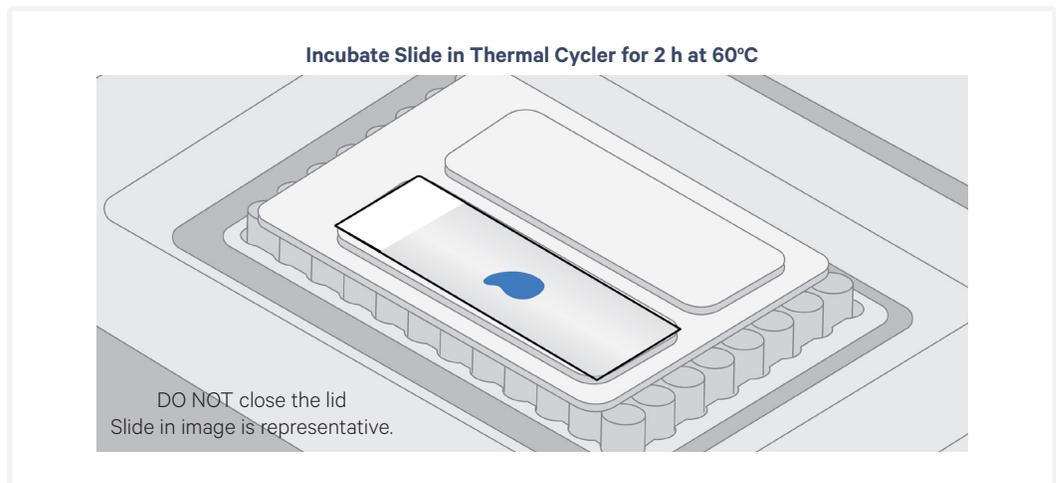
- a. Retrieve slides with tissue sections from desiccator after overnight drying.
- b. Place slides in a Section Dryer Oven and incubate uncovered at **60°C** for **2 h**. Keep the oven lid closed during incubation.



Alternatively, place a Low Profile Thermocycler Adapter on a thermal cycler set at **60°C**. Place slide on the Low Profile Thermocycler Adapter with the tissue side facing up and incubate **2 h** at **60°C**.



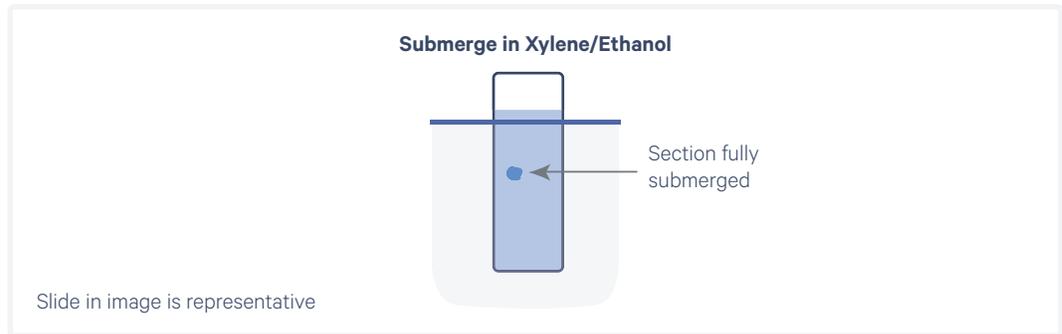
DO NOT close the thermal cycler lid.



- c. Remove slides from the oven or thermal cycler and allow to cool down to room temperature for **5 min**.



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



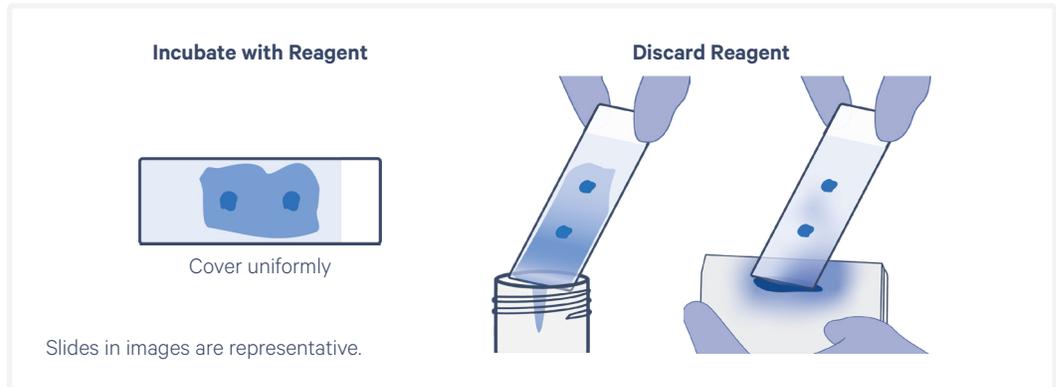
- d.** Gently immerse slides in Xylene Jar 1. Secure the jar cap to prevent xylene loss.
- e.** Incubate for **10 min.**
- f.** Gently immerse slide in Xylene Jar 2 and incubate for **10 min.**
- g.** Gently immerse slides in the 100% Ethanol Jar 1 for **3 min.**
- h.** Gently immerse slides in the 100% Ethanol Jar 2 for **3 min.**
- i.** Gently immerse slides in the 96% Ethanol Jar 1 for **3 min.**
- j.** Gently immerse slides in the 96% Ethanol Jar 2 for **3 min.**
- k.** Gently immerse slides the 70% Ethanol Jar for **3 min.**
- l.** Gently immerse slides in Water Beaker 1 and incubate for **20 sec.**
- m.** Proceed **immediately** to H&E Staining & Coverslipping.



*DO NOT let the slides dry.*

## 1.3 H&E Staining

- a. Place on a flat, clean, nonabsorbent work surface. Some residual droplets may remain.
- b. Add **1 ml** Hematoxylin per slide to uniformly cover all tissue sections.
- c. Incubate **3 min** at **room temperature**.
- d. Discard reagent by draining and/or holding slides at an angle with the bottom edge in contact with a laboratory wipe.



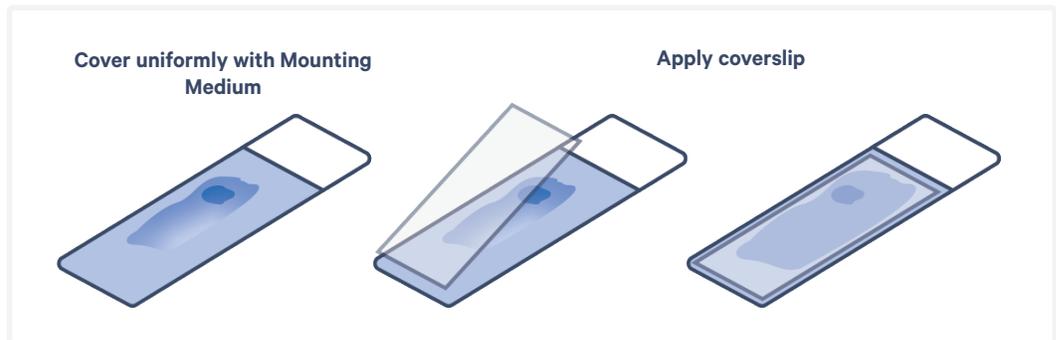
- e. Immerse slides 5x in Water Beaker 2.
- f. Immerse slides 15x in Water Beaker 3.
- g. Immerse slides 15x in Water Beaker 4.
- h. Wipe excess liquid from the back of the slides without touching the tissue section.
- i. Place on a flat, clean, nonabsorbent work surface. Some droplets may remain.
- j. Add **1 ml** Bluing Buffer per slide to uniformly cover all tissue sections. Incubate **1 min** at **room temperature**.
- k. Discard reagent by draining and/or holding slides at an angle with the bottom edge in contact with a laboratory wipe.
- l. Immerse slides 15x in Water Beaker 5.
- m. Wipe excess liquid from the back of the slides without touching the tissue section. Place on a flat, clean, nonabsorbent work surface. Some droplets may remain.
- n. Gently immerse the slides in alcoholic Eosin solution in separate 50 ml conical tubes. Incubate **2 min** at **room temperature**. **DO NOT** use diluted Eosin. Discard reagent by draining and/or holding slides at an angle with the bottom edge in contact with a laboratory wipe.
- o. Immerse slides for **30 sec** in Water Beaker 6.
- p. Immerse slides 10x in Water Beaker 7.
- q. Wipe excess liquid from the back of the slides without touching the tissue section.



*DO NOT* air dry the slides.

## 1.4 Coverslipping

- a. Place slides on a flat, clean, nonabsorbent work surface. Some residual droplets may remain.
- b. Using a **wide-bore** pipette tip, add **100–150 µl** Mounting Medium to uniformly cover all tissue sections on slides.
- c. Apply the coverslip at an angle on one end of the slides. Slowly lower the coverslip, without introducing bubbles. Allow glycerol to spread and settle.
- d. If needed, remove any large excess of Mounting Medium by carefully wicking away from the edge of the coverslip with a laboratory wipe. Be careful not to move coverslip and disturb the tissue.
- e. Once coverslipping is complete, **immediately** proceed with imaging or store slides laying flat in a slide mailer or 50-ml conical at **4°C** in the dark for up to **24 h**. Ensure slides do not come in contact with one another.

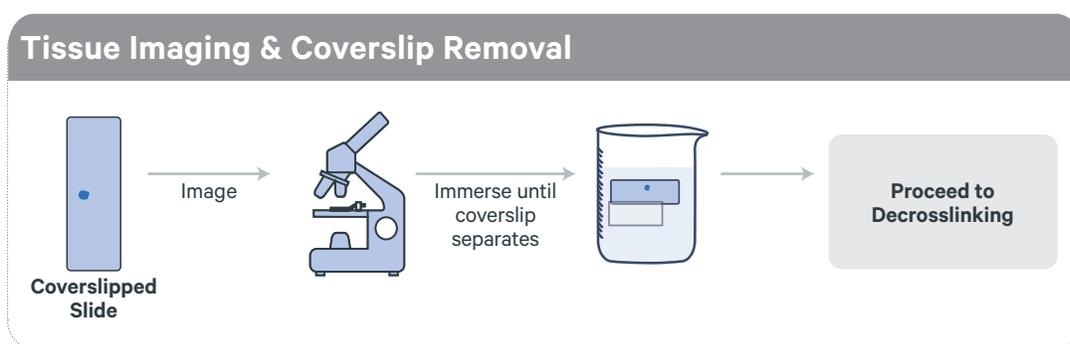


*DO NOT let the attached coverslip dry.  
DO NOT use Cytoseal or nail polish for securing the coverslip.*

## 2. Tissue Imaging

### 2.0 Overview

This chapter provides guidance on imaging tissue slides containing H&E stained FFPE sections and coverslip removal.



### 2.1 Imaging System Recommendations

The following table shows imaging systems used by 10x Genomics in the development of this protocol. Any equivalent imaging system can be used as an alternative.

Supplier	Model	Configuration
Thermo Fisher Scientific	EVOS M7000	Inverted
Leica	Aperio Versa 8	Upright
	Leica DMI8	Inverted
MetaSystems	Metafer	Upright
Nikon	Nikon Eclipse Ti2	Inverted
BioTek	Cytation 7	Inverted or Upright
Keyence	Keyence BZX800	Inverted
Olympus	VS200	Upright
Zeiss	Imager.Z2	Upright

Brightfield Recommended Configuration
Color camera (3 x 8 bit, 2,424 x 2,424 pixel resolution)
White balancing functionality
2.18 µm/pixel minimum capture resolution
Exposure times 2-10 milli sec

## 2.2 Imaging

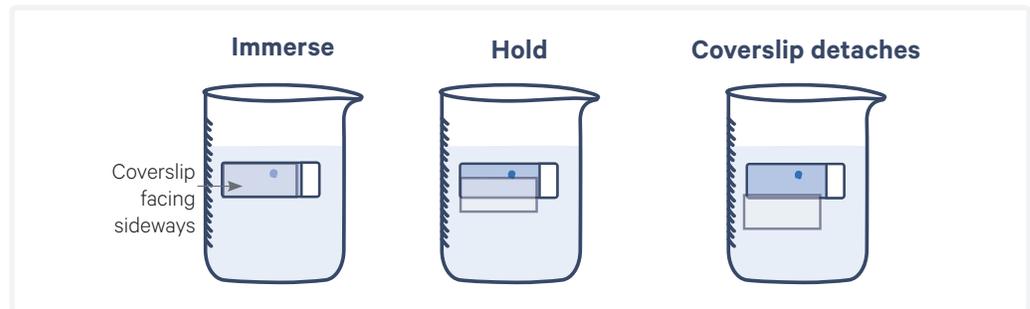
- a. Image each tissue section individually at the desired magnification using brightfield imaging settings.
- b. Consult the Visium CytAssist Spatial Gene Expression for FFPE Imaging Guidelines Technical Note (CG000521) for additional information.
- c. After imaging, proceed **immediately** to Coverslip Removal.

## 2.3 Coverslip Removal

- a. Dispense **800 ml** Milli-Q water in a beaker. Up to 10 slides may be processed using this beaker.
- b. Immerse slides sideways/horizontal in the beaker containing **800 ml** water with the coverslipped surface fully sideways.
- c. Hold slides in water until the coverslip slowly separates away from the slide.



To avoid damaging the tissue sections or causing tissue detachment, **DO NOT** move the slide up and down, shake forcibly or manually move the coverslip.



- d. Gently immerse slides 30x in water to ensure all Mounting Medium is removed.
- e. Wipe the back of the slide with a laboratory wipe. Place on a flat, clean, nonabsorbent work surface and air dry.
- f. Incubate slide on the Low Profile Thermocycler Adapter with the thermal cycler lid open for **3 min** at **37°C** to dry the slide.
- g. Proceed **immediately** to Decrosslinking or store slides in a slide mailer or 50-ml conical at **4°C** in the dark for up to **2 weeks**. Ensure slides do not touch one another.



## 3. Decrosslinking for H&E Stained Sections

### 3.0 Overview

This chapter provides guidance on performing decrosslinking to release RNA that was sequestered by the formalin fixation. Ensure that the coverslip is removed before starting decrosslinking.

This chapter is also the entry point for slides with H&E stained tissues that have had their hardset coverslips removed as described in the Visium CytAssist Spatial Gene Expression for FFPE - Tissue Preparation Guide (Document CG000518). These slides are deparaffinized during the hardset coverslip removal protocol.

Once sections have been decrosslinked, step 1 (Probe Hybridization) of Visium CytAssist Spatial Gene Expression for FFPE User Guide (CG000495) should be immediately performed.



### 3.1 Preparation - Buffers

For Decrosslinking						
Items	Preparation & Handling					
<input type="checkbox"/> 0.1 N HCl	Prepare 0.1 N HCl using nuclease-free water.					
<input type="checkbox"/> Diluted Decrosslinking Buffer	Thaw Decrosslinking Buffer at room temperature. Vortex and centrifuge briefly. Vortex and centrifuge briefly after preparing Diluted Decrosslinking Buffer. Store excess stock buffer at 4°C.					



6.5 mm Gaskets						
Diluted Decrosslinking Buffer	Stock	Final	1X (µl)	2X+ 10% (µl)	4X+ 10% (µl)	
<b>Decrosslinking Buffer</b>	10X	1X	25	55	110	
<b>Nuclease-free Water</b>	-		225	495	990	
<b>Total</b>	-	-	<b>250</b>	<b>550</b>	<b>1,100</b>	

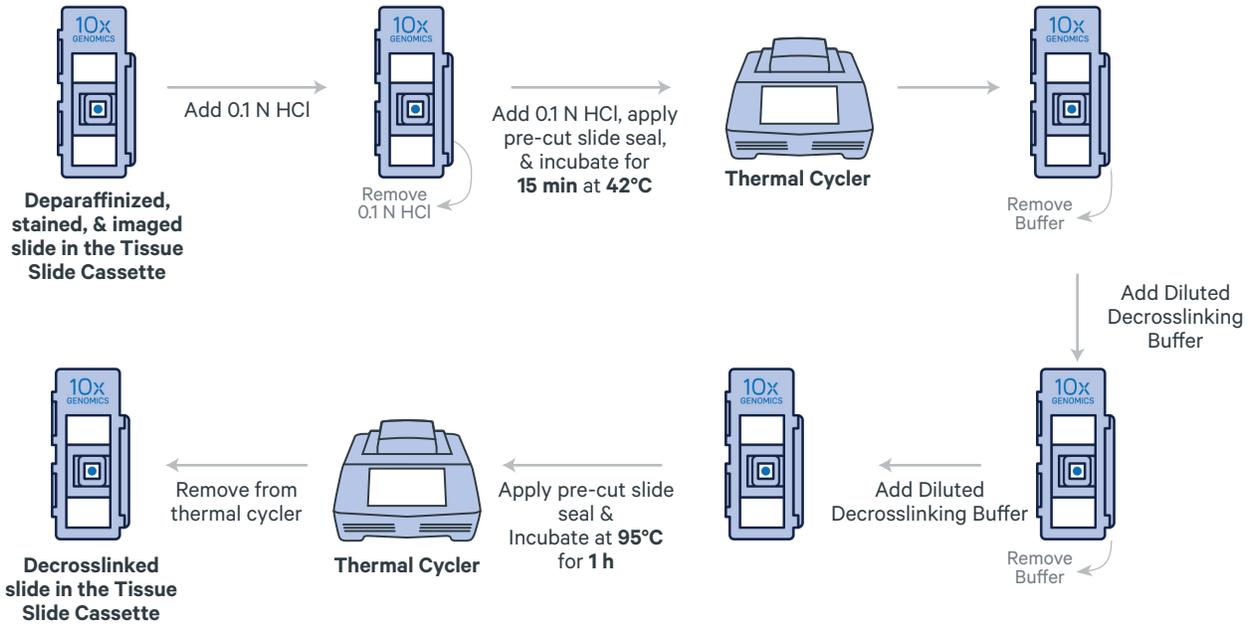
  



11 mm Gaskets						
Diluted Decrosslinking Buffer	Stock	Final	1X (µl)	2X+ 10% (µl)	4X+ 10% (µl)	
<b>Decrosslinking Buffer</b>	10X	1X	50	110	220	
<b>Nuclease-free Water</b>	-		450	990	1,980	
<b>Total</b>	-	-	<b>500</b>	<b>1,100</b>	<b>2,200</b>	

## Protocol Overview

### Destaining and Decrosslinking for H&E ~1.5 h



Once the sections have been decrosslinked, proceed directly to step 1 (Probe Hybridization) of the Visium CytAssist Spatial Gene Expression for FFPE User Guide (CG000495)

### 3.2 Destaining

■ denotes volumes for 6.5 mm gaskets and ▲ denotes volumes for 11 mm gaskets

- a. Place a Low Profile Thermocycler Adapter in the thermal cycler. Prepare the thermal cycler with the following incubation protocol and start the program.

Lid Temperature	Reaction Volume	Run Time
42°C (lid may be set to lowest setting if instrument does not enable 42°C)	100 µl	15 min

Step	Temperature	Time
Pre-equilibrate	42°C	Hold
Destaining	42°C	00:15:00
Hold	22°C	Hold

- b. Place the slide in the Visium CytAssist Tissue Slide Cassette.



See *Tips & Best Practices* for assembly instructions. Practice assembly with a blank slide.

- c. Add ■150 µl or ▲300 µl 0.1 N HCl along the side of the wells to uniformly cover the tissue sections, without introducing bubbles. Tap cassette gently to ensure uniform coverage.

- d. Remove HCl from the wells.

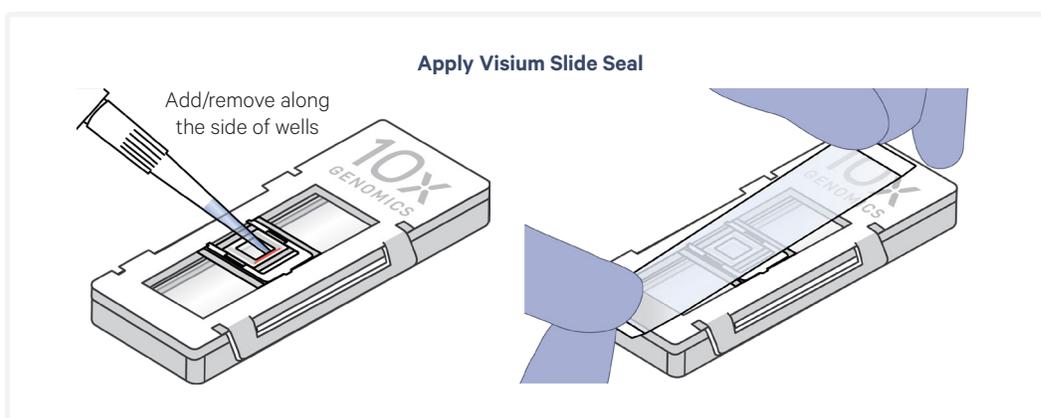


- e. Add ■100 µl or ▲200 µl 0.1 N HCl along the side of the wells to uniformly cover the tissue sections, without introducing bubbles. Tap cassette gently to ensure uniform coverage.

- f. Apply pre-cut slide seal on cassette and place the cassette on the Low Profile Thermocycler Adapter at 42°C.

- g. Close the thermal cycler lid. Skip Pre-equilibrate step to initiate Destaining.

- h. Remove the cassette from the Low Profile Thermocycler Adapter and place on a flat, clean work surface. Some color remaining in the tissue after Destaining is normal.

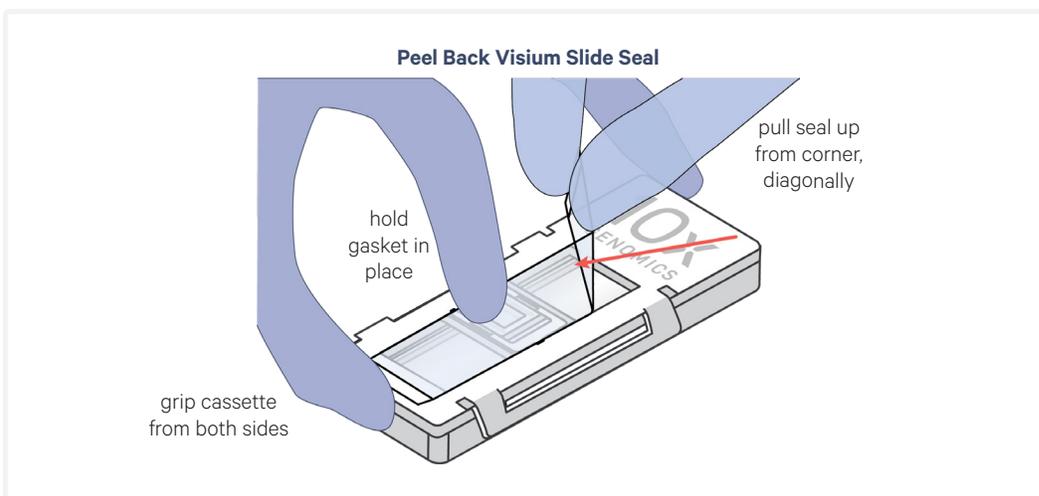


### 3.3 Decrosslinking

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

Lid Temperature	Reaction Volume	Run Time
95°C	100 µl	60 min
Step	Temperature	Time
Pre-equilibrate	95°C	Hold
Decrosslinking	95°C	00:60:00
Cooling	22°C	00:10:00
Hold	22°C	Hold

- b. Peel back slide seal and using a pipette, remove all the HCl from the well corners.



- c. Add **150 µl or 300 µl** Diluted Decrosslinking Buffer along the side of the wells.
- d. Remove Diluted Decrosslinking Buffer from the wells.
- e. Add **100 µl or 200 µl** Diluted Decrosslinking Buffer along the side of the wells.
- f. Re-apply slide seal on the cassette and place the cassette on the Low Profile Thermocycler Adapter at **95°C**.
- g. Close the thermal cycler lid. Skip Pre-equilibrate step and initiate Decrosslinking.
- h. Proceed **immediately** to Visium CytAssist Spatial Gene Expression for FFPE User Guide (CG000495).



## Troubleshooting

### Notes

#### Tissue Detachment

- Ensure compatible blank slides are used to minimize tissue detachment. Refer to Visium CytAssist Tested Slides.

#### Bubbles

- Avoid bubble formation during coverslipping. Introduction of bubbles can be mitigated by applying the coverslip at an angle and slowly lowering it onto the slide, allowing air to escape. Briefly centrifuge mounting medium to remove bubbles before use. Avoid introducing bubbles when pipetting mounting medium onto slide.

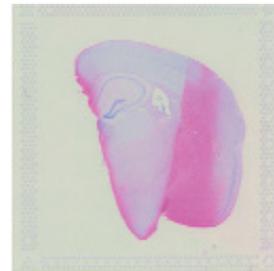


**Bubbles may cause blackening of tissue**

Slide in image is representative

#### Uneven Staining

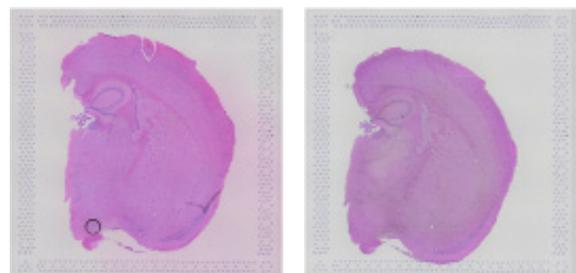
- Ensure fresh staining reagents are applied to the tissue uniformly and adequate washes are performed. A gentle tap may help reagents spread uniformly. Consider filtering hematoxylin.



Slide in image is representative

#### Incorrect Staining Protocol

- Ensure that the correct staining protocol with fresh reagents was followed. If problem persists, inconsistent staining might be attributed to improper deparaffinization. In this case, use fresh reagents.



**Incorrect staining protocol may result in poor staining performance.**

Slide in image is representative

## Document Revision Summary

<b>Document Number</b>	CG000520
<b>Title</b>	Visium CytAssist Spatial Gene Expression for FFPE – Deparaffinization, H&E Staining, Imaging & Decrosslinking
<b>Revision</b>	Rev C
<b>Revision Date</b>	April 2024
<b>General Changes</b>	<p>Updated for general minor consistency of language and terms throughout</p> <ul style="list-style-type: none"><li>• Updated magnetic separator part number on page 4.</li><li>• Added PTC Tempo and VeritiPro thermal cyclers to list on page 4.</li><li>• Updated Low Profile Thermocycler Adapter image for clarity and added additional guidance for adapter removal on page 4 and 5.</li><li>• Updated workflow overview on page 5 for clarity.</li><li>• Add Millipore Sigma part number HT110332 as a validated Eosin on page 6.</li></ul>
<b>Specific Changes</b>	<ul style="list-style-type: none"><li>• Clarified frosted area descriptions on page 16.</li><li>• Updated protocol overview on page 18.</li><li>• Updated slide illustration on page 17 and 21.</li><li>• Converted photos to technical illustrations on pages 9-15, 20-23, and 29-30 for clarity.</li><li>• Removed references to archived IF slides on pages 26, 28 and 29.</li></ul>

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