Visium Spatial Gene Expression for FFPE –

Deparaffinization, H&E Staining, Imaging & Decrosslinking

Introduction

The Visium Spatial Gene Expression for FFPE is designed to measure mRNA in tissue sections derived from formalin fixed & paraffin embedded (FFPE) tissue samples and requires a Visium Spatial 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 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 Spatial Gene Expression for FFPE - Tissue Preparation Guide (Document CG000408) for complete information on sectioning FFPE tissue blocks and placing sections on Visium Spatial slides. Ensure that tissue sections have been placed onto the appropriate slide prior to starting this Demonstrated Protocol. Consult the Visium Spatial Gene Expression for FFPE Imaging Guidelines (Document CG000436) to verify imaging settings prior to starting this Demonstrated Protocol. After completing this Demonstrated Protocol (CG000409), proceed with the Visium Spatial Gene Expression for FFPE - User Guide (CG000407).

Contents

2 Reagent Kits

3 Recommended Thermal Cyclers

3 Workflow Overview

4 Specific Reagents & Consumables

5 Tips & Best Practices

14 Protocol Steps & Timing

15 1. Deparaffinization & H&E Staining

15 1.1 Preparation - Buffers

16 Protocol Overview

18 1.2 Deparaffinization

20 1.3 H&E Staining

21 1.4 Coverslipping

22 2. Tissue Imaging

22 2.1 Imaging System Recommendations

23 2.2 Imaging

23 2.3 Coverslip Removal

24 3. Decrosslinking for H&E Stained Sections

24 3.1 Preparation - Buffers

25 Protocol Overview

26 3.2 Decrosslinking for H&E Stained sections

27 Troubleshooting

Reagent Kits

Visium Spatial Gene Expression for FFPE Reagent Kits

Refer to SDS for handling and disposal information

Visium Spatial Gene Expression Slide Kit, 16 rxns PN-1000185

Visium
Spatial Gene Expression Slide Kit
16 rxns, PN-1000185
store at ambient temperature

	#	PN
Visium Spatial Gene Expression Slide	4	2000233
*Visium Slide Seals, 40-pack	1	2000284
Visium Cassette & Gasket Assembly, 4-pack	1	2000282

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Visium Spatial Gene Expression Slide Kit, 4 rxns PN-1000188

Visium
Spatial Gene Expression Slide Kit
4 rxns, PN-1000188
store at ambient temperature

	#	PN
Visium Spatial Gene Expression Slide	1	2000233
*Visium Slide Seals, 12-pack	1	2000283
Visium Cassette & Gasket Assembly, 1-pack	1	2000281

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**Visium Slide Seals may come in varying dimensions and quantities in different lots. Check the number of slide seals in the kit. Additional seals may be required. Refer to page 13 (Visium Slide Seal Application & Removal) of this Demonstrated Protocol for instructions on how to resize seals or cut additional seals..*

Visium Tissue Section Test Slides, 4 Pack PN-1000347

Visium
Tissue Section Test Slides
4 Pack, PN-1000347
store at ambient temperature

	#	PN
Visium Tissue Section Test Slide	4	2000460

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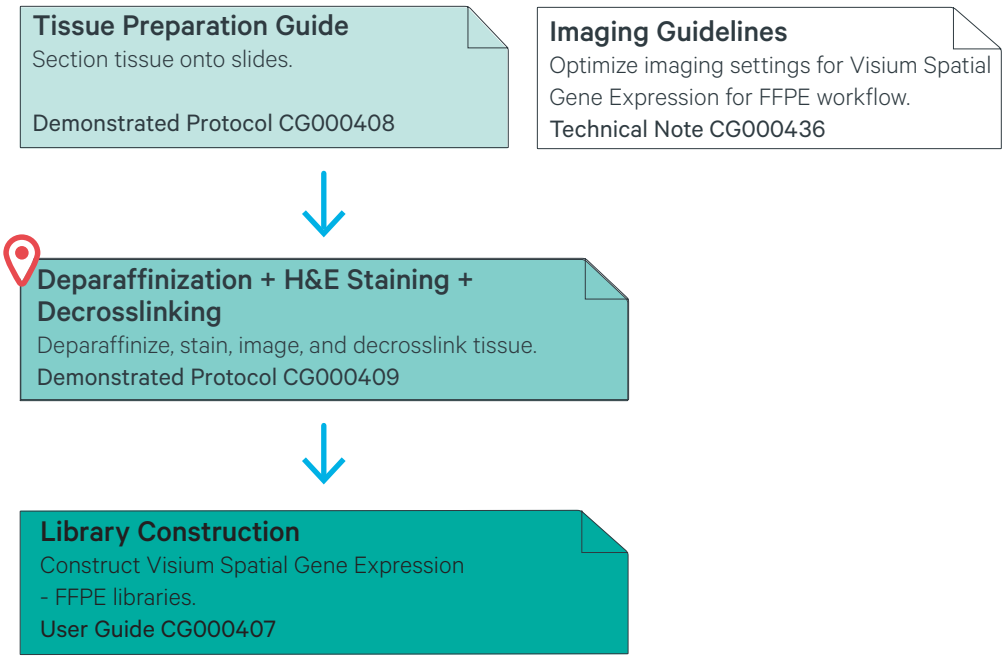
10x Genomics Accessories

Product	Part Number (Kit)	Part Number (Item)
Thermocycler Adaptor	1000194	3000380
Visium Spatial Imaging Test Slide		2000235
10x Magnetic Separator		230003
Slide Alignment Tool		3000433

Recommended Thermal Cyclers

Supplier	Description	Part Number
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Eppendorf	MasterCycler Pro (discontinued)	North America 950030010 International 6321 000.019
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler	4375786

Workflow Overview



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

Specific Reagents & Consumables

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

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 (Modified Alcoholic)	Abcam	ab246824
	Eosin Y with Phloxine 1% alcoholic solution	VWR	10143
Hemato-xylin	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 *Or any equivalent HCl*	Fisher Chemical	SA54-1
TE Buffer (pH 9.0) Or Prepare using Tris and 0.5 M EDTA	TE Buffer (pH 9.0) *Alternatively, prepare TE buffer using Tris and EDTA and adjust the pH to 9.0*	GeneMed	10-0046
	Tris Base *For preparing TE buffer (pH 9.0), alternative to Genemed product*	Fisher Scientific	BP152-500
	UltraPure 0.5 M EDTA, pH 8.0 *For preparing TE buffer (pH 9.0), alternative to Genemed product*	Thermo Fisher Scientific	15575020
	Dilute to 1.0 M HCl stock *For preparing TE Buffer (pH 9.0), alternative to Genemed product*	Millipore Sigma	258148
Staining jar/ dishes	Coplin Jar	VWR	100500-232
	Staining Dishes	VWR	25608-906
Section dryer oven	Epredia High Capacity Section Dryer *Or any equivalent product*	Fisher Scientific	A84600051
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
Sealing film	Microseal 'B' PCR Plate Sealing Film, adhesive	Bio-Rad	MSB1001

Additional Materials		
Beakers	-	-
Ultrapure/Milli-Q Water, *from Milli-Q Integral Ultrapure Water System or equivalent*	-	-

Tips & Best Practices

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

General Reagent Handling

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

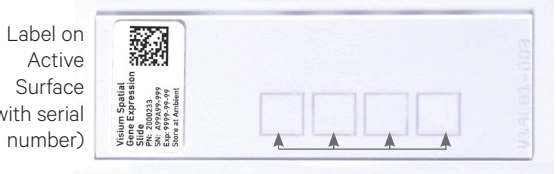
Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.

Visium Spatial Gene Expression Slide

- Visium slides include 4 Capture Areas (6.5 x 6.5 mm), each with ~5,000 unique gene expression spots.
- Each gene expression spot includes primers with a unique Spatial Barcode.
- The active surface of the slide is defined by a readable label that includes the serial number.
- The tissue sections are always placed on the active surface of the Capture Areas. For more information, consult the Visium Spatial Gene Expression for FFPE – Tissue Preparation Guide (Demonstrated Protocol CG000408).
- Always store slides in a cool, dry environment. After tissue placement, store the slides at room temperature in a low moisture environment such as a desiccator.

Visium Spatial Gene Expression Slide

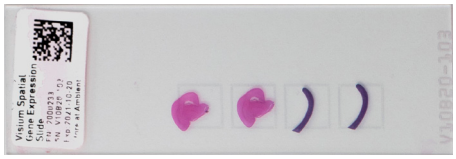


Note the serial number on the slide label will be required for downstream analysis.

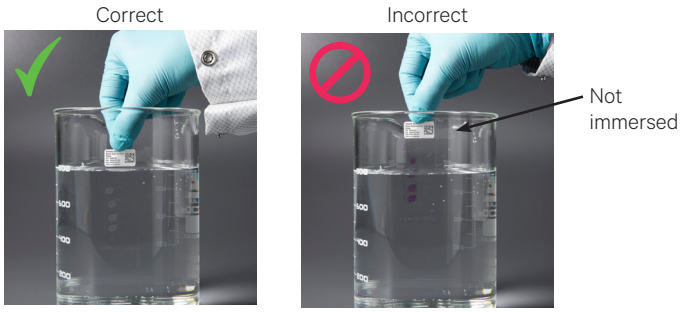
Slide Handling

- Always wear gloves when handling slides.
- Ensure that the active surface of a slide faces up and is never touched. The orientation of the label on the slide defines the active surface.
- The tissue sections should always be on the active surface of the slide. 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.
- Xylene and ethanol may cause the slide label to come off. Keep the label above the surface of the liquid when immersing in xylene and ethanol.
- Keep the slide flat on the bench when adding reagents to the active surface.
- Ensure that no absorbent surface is in contact with the reagents on the slide during incubation.

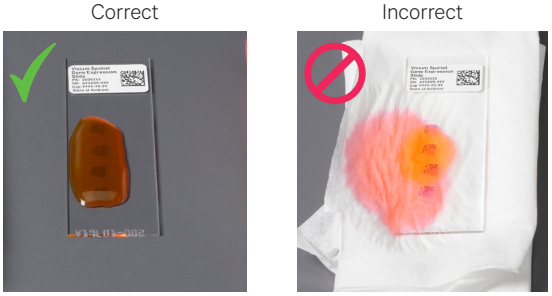
Active Surface with Tissue Sections



Immersing Slide



Reagent on Slide



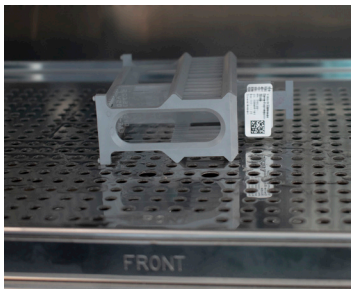
Slide Incubation Guidance

Incubation at a specified temperature

Incubation using a Section Dryer Oven:

- Place the slides in a slide drying rack.
- Close the lid when incubating the slide in the oven.

Place in a Rack



Slides in images are representative.

Incubation using a Thermal Cycler:

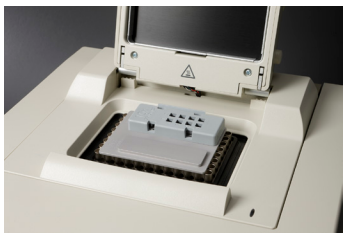
- Position a Thermocycler Adaptor on a thermal cycler that is set at the incubation temperature.
- Ensure that the Thermocycler Adaptor is in contact with the thermal cycler surface uniformly.
- When incubating a slide, position the slide on the Thermocycler Adaptor with the active surface facing up.
- Ensure that the entire bottom surface of the slide is in contact with Thermocycler Adaptor.
- When incubating a slide encased in a Visium Cassette, place the assembled unit on the Thermocycler Adaptor with the wells facing up. The Visium Cassette should always be sealed when in the Thermocycler Adaptor.

Place Thermocycler Adaptor

Incubate Slide

DO NOT close lid

Incubate Assembled Visium Cassette



Close lid

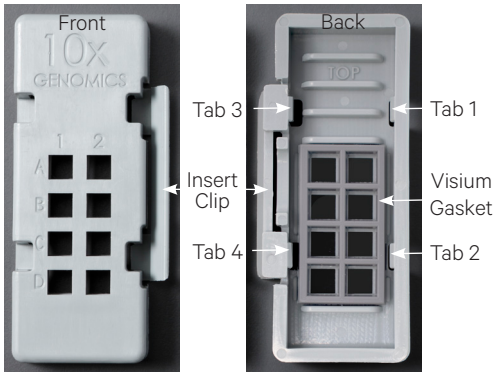
Incubation at room temperature

- Place the slide/Visium 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.

Visium Cassette

- The Visium Cassette encases the slide and creates leakproof wells for adding reagents.
- Place the slides in the Visium Cassette only when specified.
- The Visium Cassette includes a removable Visium Gasket.
- An Insert Clip and four tabs at the back of the Visium Cassette are used for holding the slide in the cassette, as shown.
- The removable Visium Gasket corresponds to the Capture Areas on the slides.
- The Visium Cassette may be assembled using the Slide Alignment Tool or manually. Instructions for both are provided in the following section.
- See Visium Cassette Assembly & Removal instructions for details.
- Ensure that the back of the Visium Cassette is facing the user prior to assembly. The active surface of the slide with tissue sections will face down such that the slide label is no longer readable.
- Practice assembly with a plain glass slide (75 x 25 x 1 mm).
- Applying excessive force to the slide may cause the slide to break.

Visium Cassette

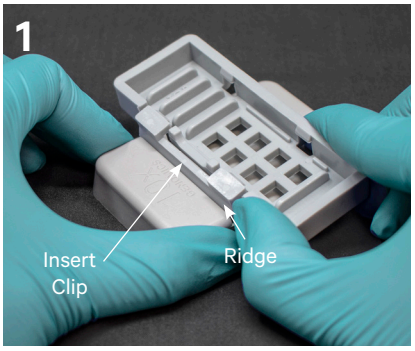


Slide Alignment Tool

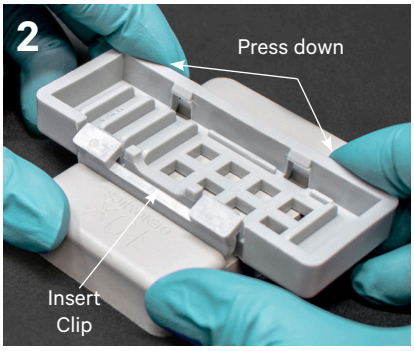
Visium Cassette Assembly

Exercise caution when handling slide edges to prevent injury.

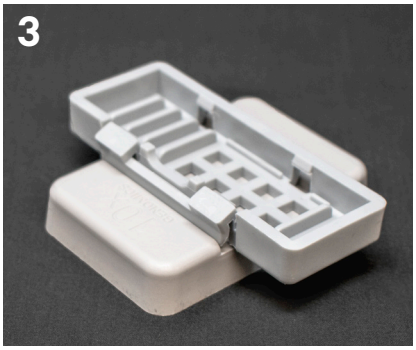
Position Visium Cassette along alignment tool ridges



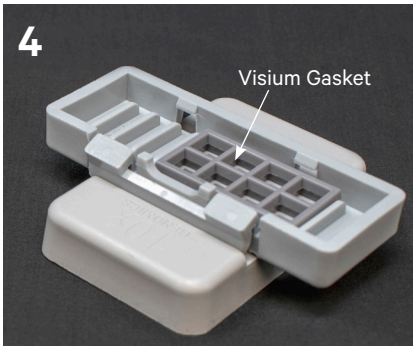
Push Insert Clip along the ridge & press Visium Cassette down



Visium Cassette secured on alignment tool

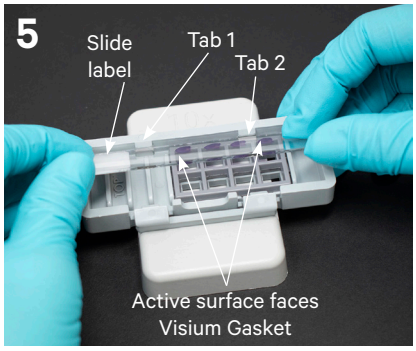


Position Visium Gasket to align with Visium Cassette cutouts

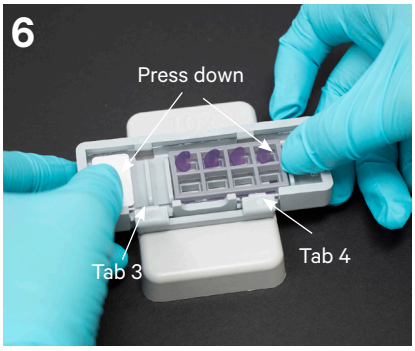


Slide insertion may push Visium Gasket out of alignment with slide cutouts. Adjust if necessary.

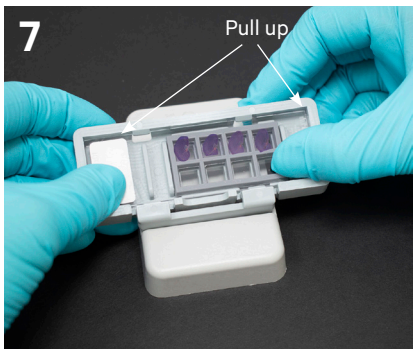
Insert long edge of slide under tabs 1 & 2; ensure slide is flush



Press slide down until it is flush with the Visium Gasket and under tabs 3 & 4



Remove Visium Cassette while pressing slide against the Visium Gasket

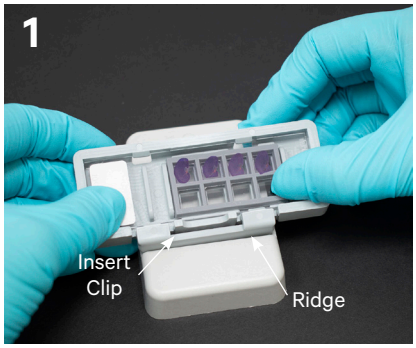


Slides in images are representative.

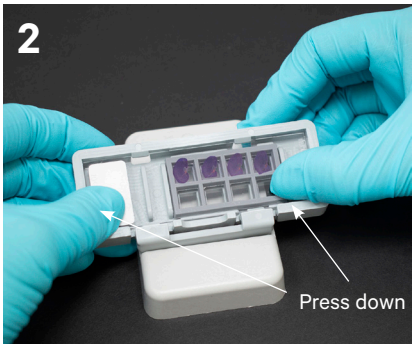
Visium Cassette Removal

Slides in images are representative.

Position Visium Cassette along alignment tool ridges

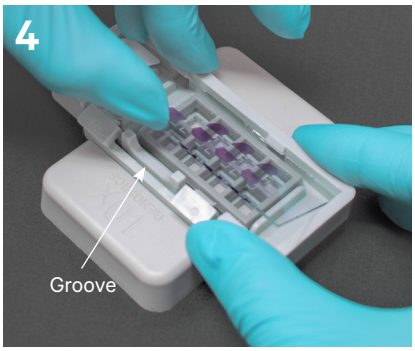


Push Insert Clip along the ridge & press down



Visium Cassette sits securely on alignment tool

Lift slide at Visium Cassette groove



Manual Visium Cassette Assembly & Removal

Assembly

a. Remove the Visium Gasket from the Visium Cassette and re-insert the Visium Gasket, ensuring that the Visium Gasket and Visium Cassette cutouts are aligned.

b. Align the label on top of the slide to the top of the Visium Cassette, as shown.

c. Insert the slide under tabs 1 and 2. Ensure that the long edge of the slide is flush with the side of the Visium Cassette.

d. Press the insert clip very firmly by applying even force on the lower part of the insert clip.

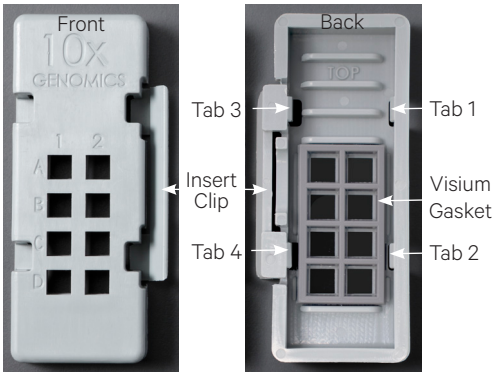
e. Place a finger in between tab 3 and the top of the cassette, and one finger between tab 4 and the bottom of the cassette.
Press down on the slide evenly until the slide is under each tab and release the insert clip.

Removal

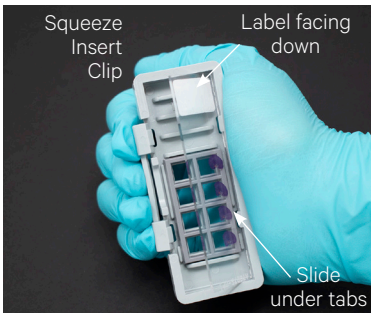
a. Press the insert clip very firmly to release the slide from the cassette.

b. Lift slide at Visium Cassette groove between tabs 3 and 4 until the slide can be removed.

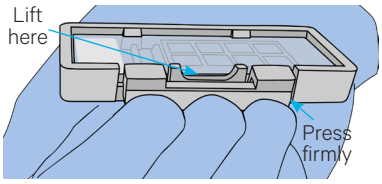
Visium Cassette



Visium Cassette Assembly



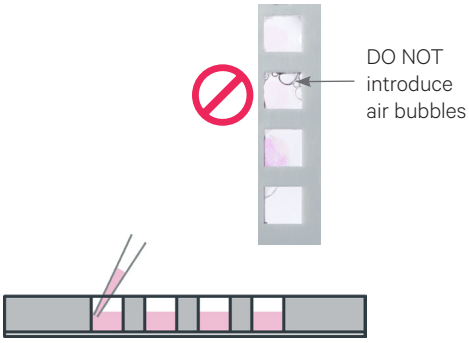
Insert Clip - Press Firmly



Reagent Addition to Wells

- Place the assembled slide in the Visium 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.
- Ensure that no bubbles are introduced in the process.

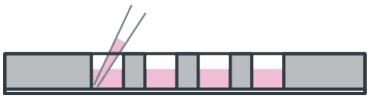
Reagent Addition/Removal



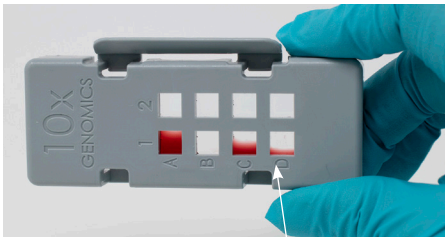
Add reagent along the slide of the wells

Reagent Removal from Wells

- Place the assembled slide in the Visium Cassette flat on a clean work surface.
- Slightly tilt the Visium 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.
- Ensure that no bubbles are introduced in the process.
- 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.



Place the pipette tip to the bottom edge of the wells



Meniscus at the bottom indicates liquid. DO NOT leave liquid behind

Visium Slide Seal Application & Removal

To generate new or resize Visium Slide Seals, use one of the provided seals (PN-2000283/2000284) as a template to cut additional seals from MicroSeal 'B' PCR Plate Sealing Film (PN-MSB1001; listed in Specific Reagents & Consumables). Contact support@10xgenomics.com if assistance is required.

Application

- Place the Visium 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 Visium Cassette and apply while firmly holding the Visium Cassette with one hand.
- Press on the Visium Slide Seal to ensure uniform adhesion.

Removal

- Place the Visium Cassette flat on a clean work surface.
- Pull on the Visium Slide Seal from the edge while firmly holding the Visium Cassette.
- Ensure that no liquid splashes out of the wells.

Visium Slide Seal Application

Tissue Detachment on Visium Slides

- Monitor section adhesion on the Visium slides throughout the workflow. For more information, consult the Visium Spatial Gene Expression for FFPE – Tissue Paration Guide (Demonstrated Protocol CG000408).
- Tissue detachment during the workflow can impact performance.

Protocol Steps & Timing

~4.5 hours

Steps		Timing	Stop & Store
Step 1 – Deparaffinization and H&E Staining			
1.2	Deparaffinization	60 min	
1.3	H&E staining	10 min	
1.4	Coverslipping	10 min	
Step 2 – Tissue Imaging			
2.2	Imaging	variable	
2.3	Coverslip removal	5 min	STOP 4°C ≤2 weeks
Step 3 – Decrosslinking for H&E Stained Sections			
3.2	Decrosslinking	60 min	

1. Deparaffinization & H&E Staining

1.0 Overview

This chapter provides guidance on deparaffinization and H&E staining of Visium 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 (CG000436) for more information.

1.1 Preparation - Buffers

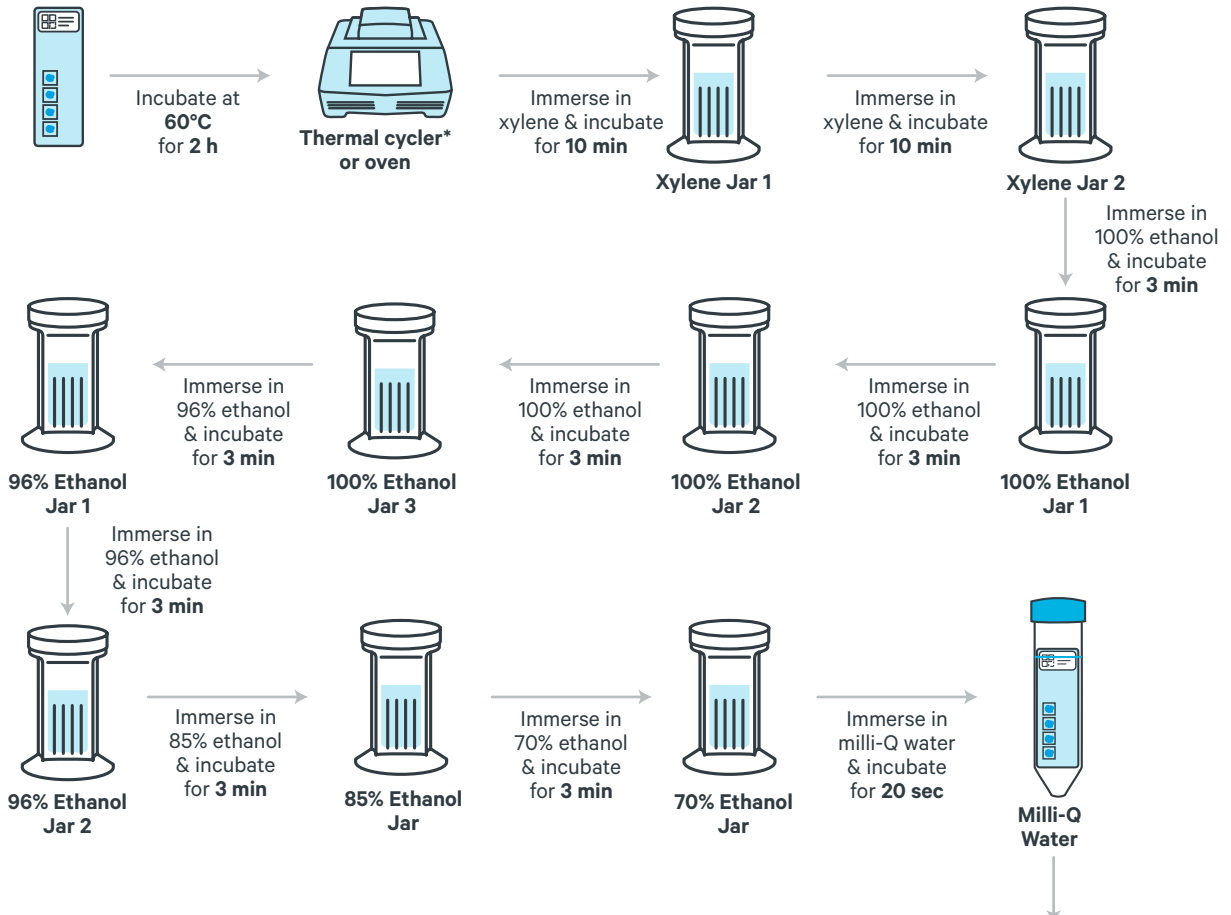
For Deparaffinization	
Items	**Preparation & Handling**
☐ Xylene	Label two coplin jars as Xylene Jar 1 and 2. Dispense 30 ml xylene in each.
☐ 100% Ethanol	Label three coplin jars as 100% Ethanol Jar 1, 2, and 3. Dispense 30 ml 100% ethanol in each.
☐ 96% Ethanol	Label two coplin jars as 96% Ethanol Jar 1 and 2. Dispense 30 ml 96% ethanol in each.
☐ 70% Ethanol	Label one coplin jar as 70% Ethanol Jar. Dispense 30 ml 70% ethanol.
☐ Milli-Q Water	Label one coplin jar as Milli-Q Water Jar. Dispense 30 ml milli-Q water. Alternatively, use a 50-ml centrifuge tube or a beaker for each individual slide.

Alternatively, a slide staining dish can also be used in place of a coplin jar. Adjust the volumes of deparaffinization solutions and water, accordingly.
Use xylene-resistant dishes for immersion in xylene. Use xylene-resistant gloves or forceps for deparaffinization. Prepare fresh reagents every week. Use fresh reagents after every 20 slides or every month (whichever comes first).

For H&E Staining	
Items	**Preparation & Handling**
☐ Milli-Q Water	Label four 1000-ml beakers as Water Beakers 1, 2, 3, and 4. Dispense the following volumes of Milli-Q water. 800 ml in Beaker 1 800 ml in Beaker 2 800 ml in Beaker 3 800 ml in Beaker 4 Dispensed volumes in each beaker can be used for two slides. Alternatively, use four 50-ml centrifuge tubes. Clean beaker with Milli-Q Water then RNase decontmination solution in between use.
☐ **Alcoholic Eosin**	30 ml in a 50-ml centrifuge tube
For Coverslipping	
Items	**Preparation & Handling**
☐ 85% Glycerol	Prepare 85% Glycerol using Nuclease-free Water. Invert mix. Prepare 150-200 µl per slide. Briefly centrifuge before using to remove bubbles.

Protocol Overview

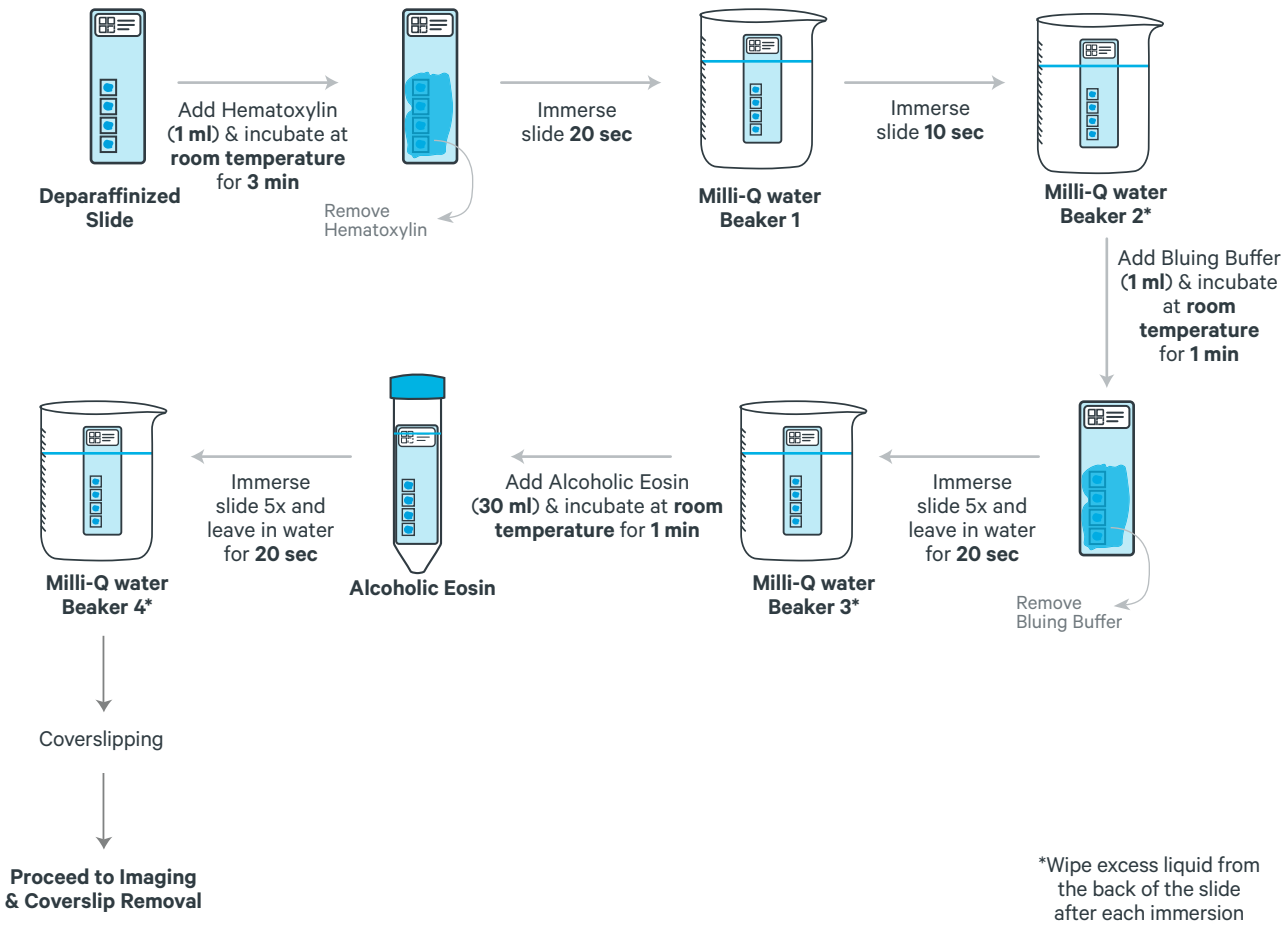
Deparaffinization ~3 h



** Keep the thermal cycler lid open during incubation, allow slide to cool to room temperature and immerse in xylene after 60°C heating step*

Protocol Overview

H&E Staining & Coverslipping ~15 min



1.2 Deparaffinization

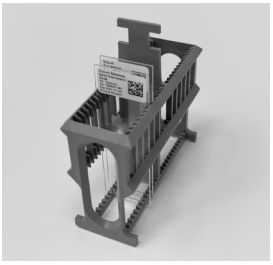
Deparaffinization steps should be performed in a fume hood due to the hazardous nature of xylene.

Visium Spatial slide contains a readable label with a serial number on the active surface of the slide. The label may come off during deparaffinization steps. In such cases, the serial number etched on the slide can be used.

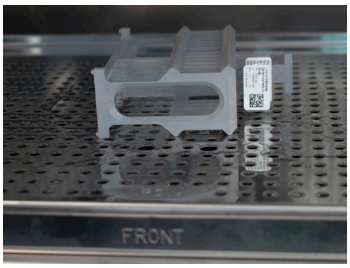
a. Retrieve the slide with tissue sections from the desiccator after overnight drying.

b. Place slides in a Section Dryer Oven and incubate uncovered at **60°C** for **2 h**. Keep the oven lid closed during incubation.

Incubation in a Section Dryer Oven



Place slide in a rack

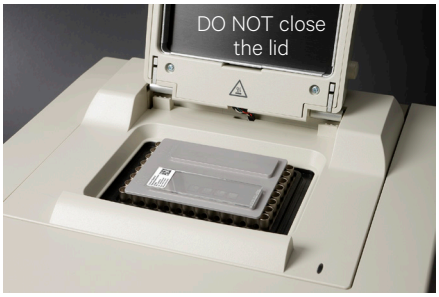


Place slide sideways and keep the oven door closed during incubation

Alternatively, place a Thermocycler Adaptor on a thermal cycler set at **60°C**. Place slide on the Thermocycler Adaptor with the active surface facing up and incubate **2 h** at **60°C**. DO NOT close the thermal cycler lid.

Incubation in a Thermal Cycler

Place Thermocycler Adaptor

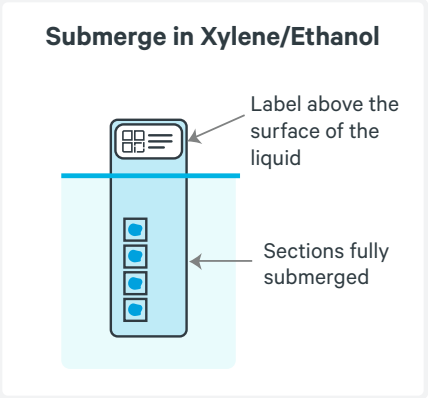


Incubate Slide for 2 h at 60°C

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

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

When immersing slides in xylene, ensure that the tissue sections are completely submerged and the xylene doesn't reach the label.



e. Incubate for **10 min**.

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

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

When immersing slides in ethanol, ensure that the tissue sections are completely submerged and the ethanol doesn't reach the label.

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

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

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

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

l. Gently immerse slide in the 85% Ethanol Jar for **3 min**.

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

n. Gently immerse slide in the water in the tube/beaker and incubate for **20 sec**.

o. Proceed **immediately** to H&E Staining & Coverslipping. *DO NOT let the slides dry.*

1.3 H&E Staining

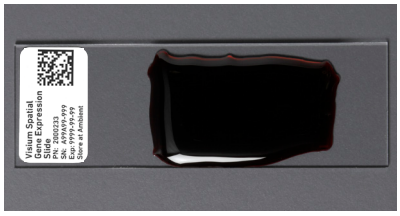
a. Place on a flat, clean, non-absorbent work surface. Some residual droplets may remain.

b. Add **1 ml** Hematoxylin to uniformly cover all tissue sections on the slide.

c. Incubate **3 min** at **room temperature**.

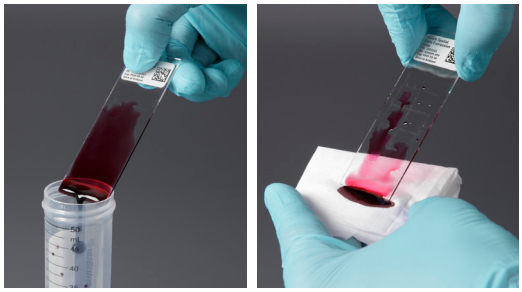
d. Discard reagent by draining and/or holding the slide at an angle with the bottom edge in contact with a laboratory wipe.

Incubate with Reagent



Cover uniformly

Discard Reagent



Slides in images are representative.

e. Gently immerse the slide for **20 sec** in the water in Beaker 1.

f. Gently immerse the slide for **10 sec** in the water in Beaker 2.

g. Wipe excess liquid from the back of the slide without touching the tissue section.

h. Place on a flat, clean, non-absorbent work surface. Some droplets may remain

i. Add **1 ml** Bluing Buffer to uniformly cover all tissue sections. Incubate **1 min** at **room temperature**.

j. Discard reagent by draining and/or holding the slide at an angle with the bottom edge in contact with a laboratory wipe.

k. Gently immerse the slide 5x in the water in Beaker 3 and leave the slide in the water for **20 sec**.

l. Wipe excess liquid from the back of the slide without touching the tissue section. Place on a flat, clean, non-absorbent work surface. Some droplets may remain.

m. Gently immerse ths slide in alcoholic Eosin solution in a 50-ml centrifuge tube. Incubate **1 min** at **room temperature**. DO NOT use diluted Eosin. Discard reagent by draining and/or holding the slide at an angle with the bottom edge in contact with a laboratory wipe.

n. Gently immerse the slide 5x in the water in Beaker 4 and leave the slide in the water for **20 sec**.

o. Wipe excess liquid from the back of the slide without touching the tissue section. *DO NOT air dry the slide.*

1.4 Coverslipping

a. Place slide on a flat, clean, non-absorbent work surface. Some residual droplets may remain.

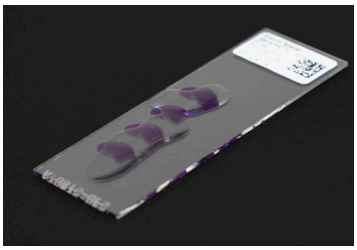
b. Using a **wide-bore** pipette tip, add **150-200 µl** 85% Glycerol to uniformly cover all tissue sections on the slide.

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

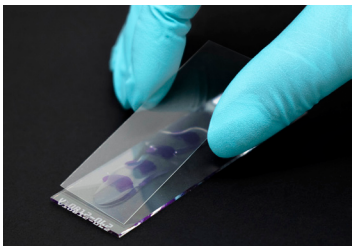
d. If needed, remove any large excess of glycerol by carefully wicking away from the edge of the coverslip with a laboratory wipe. Be careful not to move coverslip and disturb the tissue.

e. Once coverslipping is complete, **immediately** proceed with imaging.
DO NOT let the attached coverslip dry.
DO NOT use Cytoseal or nail polish for securing the coverslip.

Cover uniformly with 85% Glycerol



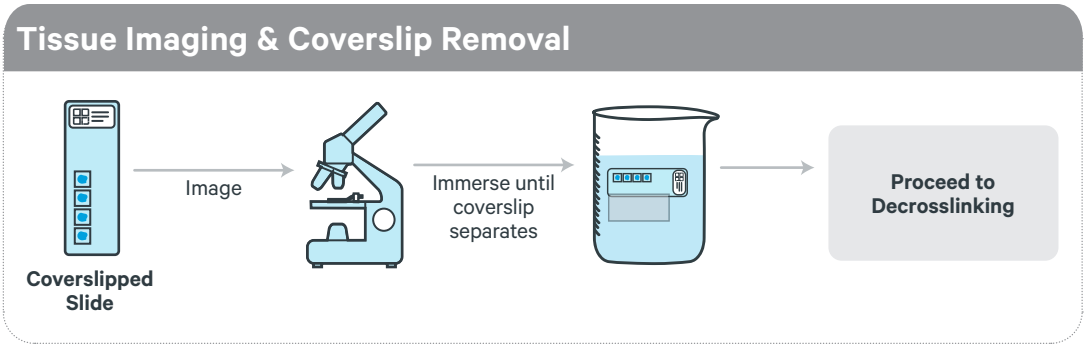
Apply coverslip



2. Tissue Imaging

2.0 Overview

This chapter provides guidance on imaging Visium 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

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 all Capture Areas individually at the desired magnification using brightfield imaging settings. Ensure that fiducial frames are captured.

b. Consult the Visium Spatial Gene Expression for FFPE Imaging Guidelines Technical Note (CG000436) for additional information.

c. After imaging, proceed **immediately** to the Coverslip Removal.

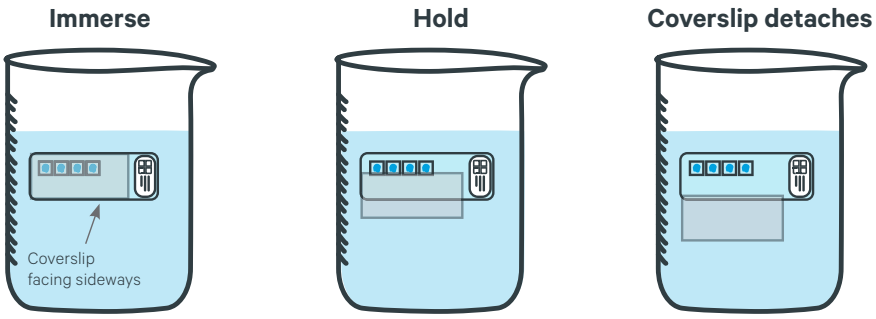
2.3 Coverslip Removal

a. Dispense **800 ml** Milli-Q water in a beaker.

b. Remove the coverslip immediately after imaging is complete.

c. Immerse the slide sideways/horizontal in the beaker containing **800 ml** water with the coverslipped surface fully sideways.

d. Hold the slide in water until the coverslip slowly separates away from the slide.
To avoid damaging the tissue sections and Capture Areas or causing tissue detachment, DO NOT move the slide up and down, shake forcibly or manually move the coverslip.



e. Gently immerse 15x in the water to ensure all glycerol is removed.

f. Wipe the back of the slide with a laboratory wipe. Place on a flat, clean, non-absorbent work surface and air dry.

g. Incubate slide on the Thermocycler Adaptor with the thermal cycler lid open for **3 min** at **37°C**.

STOP

h. Proceed **immediately** to Decrosslinking or store in a slide mailer with a desiccator or 50-ml tube at **4°C** for up to **2 weeks**.

3. Decrosslinking for H&E Stained Sections

3.0 Overview

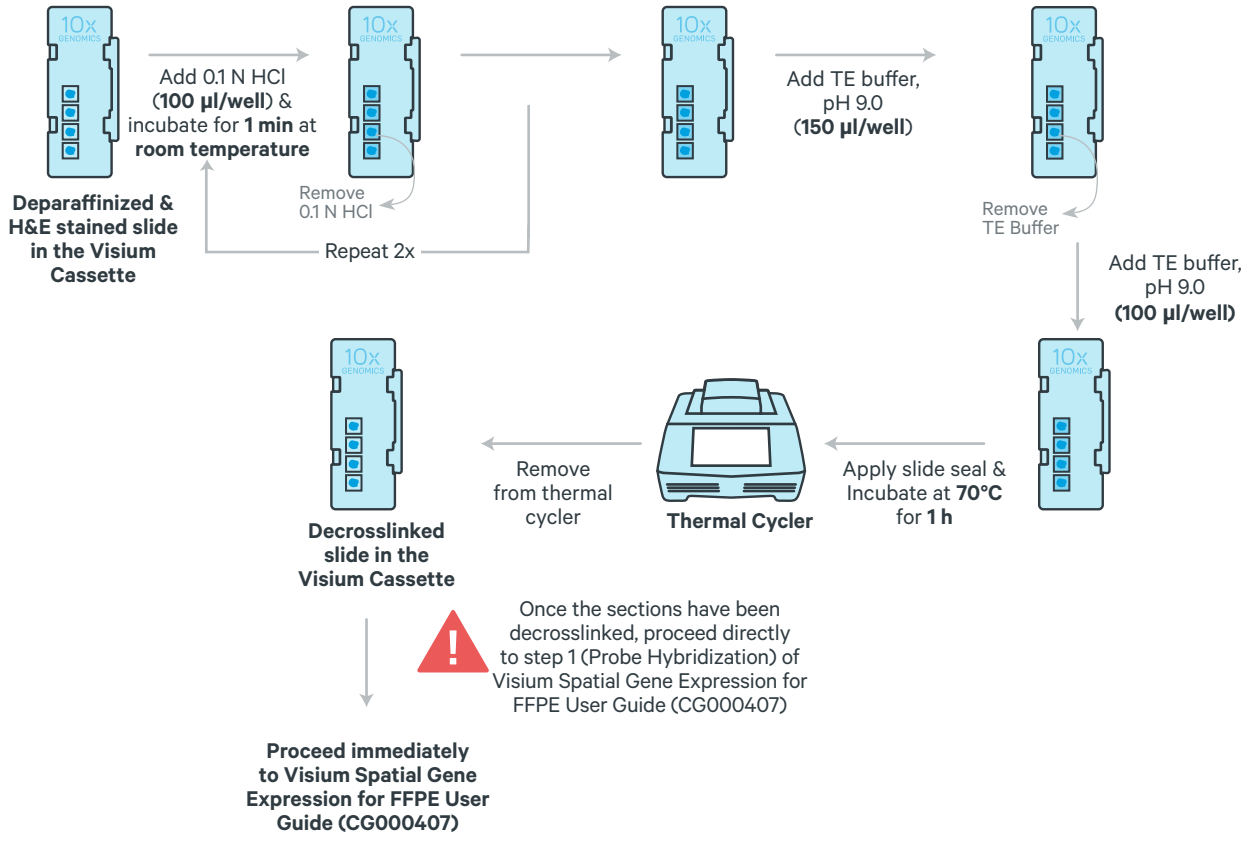
This chapter provides guidance on performing decrosslinking to release RNA that was sequestered by the formalin fixation. Ensure that the coverslip is removed before starting decrosslinking. Once the sections have been decrosslinked, step 1 (Probe Hybridization) of Visium Spatial Gene Expression for FFPE User Guide (CG000407) should be immediately performed.

3.1 Preparation - Buffers

For Decrosslinking	
Items	**Preparation & Handling**
☐ TE Buffer, pH 9.0 Or	
☐ Prepare TE Buffer, pH 9.0	**TE Buffer Preparation** (Prepare fresh and maintain at room temperature) • Dissolve 1.21 g Tris base in 950 ml nuclease-free water. • Adjust the pH to 9.0 with 1.0 M HCl. • Add 2 ml of 0.5 M EDTA and adjust the pH to 9.0 using 1.0 M HCl. • Bring the volume to 1000 ml using nuclease-free water.
☐ 0.1 N HCl	

Protocol Overview

Decrosslinking for H&E Stained Sections ~1 h



3.2 Decrosslinking for H&E Stained sections

a. Place a Thermocycler Adaptor in the thermal cycler. Prepare the thermal cycler with the following incubation protocol and start the program.

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

Lid Temperature	Reaction Volume	Run Time
70°C	-	60 min
Step	**Temperature**	**Time**
Pre-equilibrate	70°C	Hold
Decrosslinking	70°C	00:60:00
Hold	22°C	Hold

TIPS

c. Place the slide in the Visium Cassette.
See Tips & Best Practices for assembly instructions. Practice assembly with a blank slide.

d. Add **100 µl** 0.1 N HCl along the side of the wells to uniformly cover the tissue sections, without introducing bubbles. Tap Visium Cassette gently to ensure uniform coverage.

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

f. Using a pipette, remove all the HCl from the well corners.

g. Repeat d-f 2x for a total of 3 washes with 0.1N HCl.

h. Add **150 µl** TE Buffer (pH 9.0) along the side of the wells.

i. Remove all TE Buffer from the wells.

j. Add **100 µl** TE Buffer (pH 9.0) along the side of the wells.

k. Apply Visium Slide Seal on the Visium Cassette and place the cassette on the Thermocycler Adaptor at **70°C**.

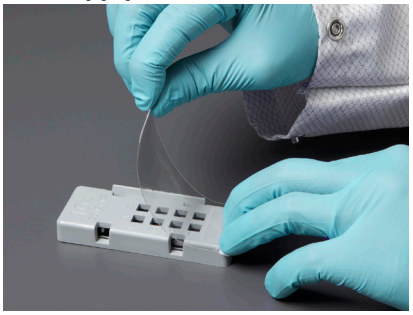
l. Close the thermal cycler lid. Skip Pre-equilibrate step and initiate Decrosslinking.

m. Once the Decrosslinking is complete, remove the cassette from the Thermocycler Adaptor and place on a flat, clean work surface.

n. Proceed **immediately** to Visium Spatial Gene Expression for FFPE User Guide (CG000407).

Add Reagent

Apply Visium Slide Seal



Troubleshooting

STEP	Notes
Tissue Detachment	• If tissue detachment is observed during the workflow, contact support@10xgenomics.com.

Document Revision Summary

Document Number	CG000409
Title	Visium Spatial Gene Expression for FFPE – Deparaffinization, H&E Staining, Imaging & Decrosslinking
Revision	Rev B to Rev C
Revision Date	June 2022
General Changes	Updated for general minor consistency of language and terms throughout
Specific Changes	• Updated to include Protocol Steps and Timing (page 14) • Updated Deparaffinization workflow (page 16, 19) • Updated beaker cleaning recommendation (page 16)

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