Visium CytAssist Spatial Gene Expression for Fresh Frozen – Methanol Fixation, H&E Staining, Imaging & Destaining

Introduction

The Visium CytAssist Spatial Gene Expression for Fresh Frozen protocol is designed to analyze mRNA in tissue sections derived from fresh frozen (FF) tissue samples. The Visium CytAssist instrument requires a glass slide with intact tissue sections as input. This protocol outlines methanol fixation, Hematoxylin & Eosin (H&E) staining, imaging, and destaining of fresh frozen tissue for use with the 10x Genomics Visium CytAssist Spatial Gene Expression assay. Though downstream reagent kits mentioned in the User Guide include FFPE in their titles, they will be used for these fresh frozen tissue sections.

Additional Guidance

Consult the Visium CytAssist Spatial Gene Expression for Fresh Frozen - Tissue Preparation Guide (CG000636) for Tips & Best Practices on freezing, embedding, cryosectioning tissue, and placing sections on blank slides. Consult the Visium CytAssist Spatial Gene Expression Imaging Guidelines (CG000521) to verify imaging settings prior to starting this Demonstrated Protocol. After completing this Demonstrated Protocol (CG000614), proceed with the Visium CytAssist Spatial Gene Expression User Guide (CG000495) Rev C or later.

Contents

Reagent Kits

Visium Spatial Gene Expression for FFPE Reagent Kits

Refer to 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 (pre-assembled with gasket)	2	3000816
Visium CytAssist moveable Cassette, frame	2	3000813
Visium CytAssist Slide Seals, 40 pack*	1	2000284
Visium CytAssist Spatial Gene Expression Slide v2, 6.5 mm	1	2000549

10x GENOMICS

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, 40 pack*	1	2000284
Visium CytAssist Spatial Gene Expression Slide v2, 11 mm	1	2000701

10x GENOMICS

**Only these items are used in this protocol.*

10x Genomics Accessories

Product	#	Kit and Part Number	Part Number ;(Item)
Low Profile Plate Insert	2	Visium CytAssist Reagent Accessory Kit: 1000499	3000823
10x Magnetic Separator	1		120250

Recommended Thermal Cyclers

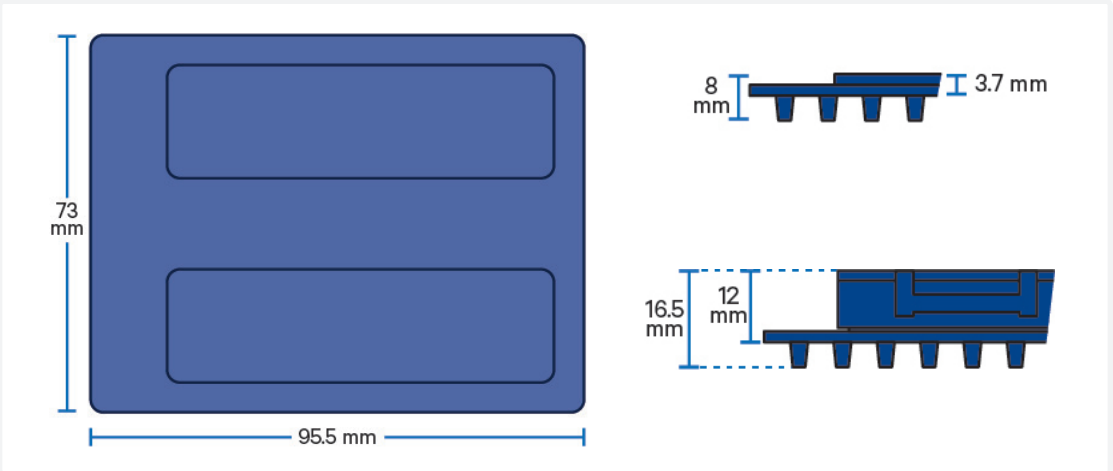
Supplier	Description	Part Number
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Eppendorf	MasterCycler Pro (discontinued)	North America 950030010 International 6321 000.019
	MasterCycler X50s	North America 6311000010
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler (discontinued)	4375786
Analytik Jena	Biometra TAdvanced 96 SG with 96-well block (silver, 0.2 mL) and gradient function	846-x-070-241

If using thermal cyclers other than the BioRad C1000, ramp rates should be adjusted for all the steps as described below:

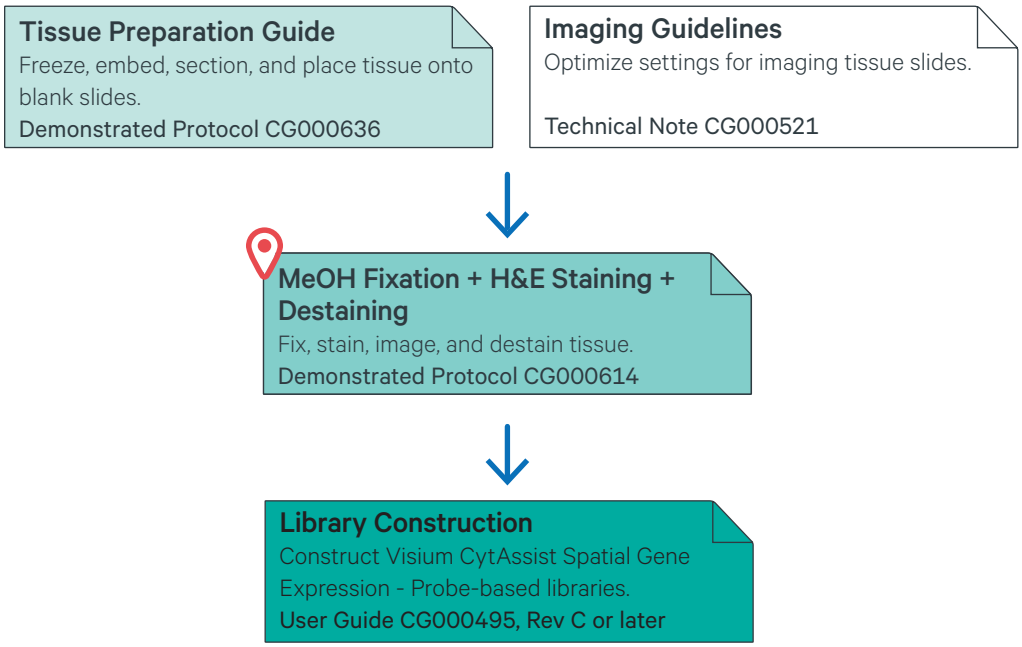
- Eppendorf MasterCycler X50s: 3°C/sec heating and 2°C/sec cooling
- Analytik Jena Biometra TAdvanced: 2°C/sec heating and cooling

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



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

Item	Alternatives/Options	Vendor	Part Number
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
Hematoxylin	Hematoxylin Solution, Mayer's	Millipore Sigma	MHS16
Methanol	Methanol, for HPLC, ≥99.9%	Millipore Sigma	34860
	Methanol, anhydrous, 99.8%	Millipore Sigma	322415
Isopropanol	2-Propanol (Isopropanol), BioReagent, for Molecular Biology, ≥99.5%	Millipore Sigma	I9516-25ML
Bluing reagent	Bluing Reagent, Dako	Agilent	CS70230-2
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
Coplin jar/ staining dishes	Coplin Jar	VWR	100500-232
	Staining Dishes	VWR	25608-906
Slide Mailers	Simport Scientific LockMailer Tamper Evident Slide Mailer	Fisher Scientific	22-038-399
	Fisherbrand 5-Place Slide Mailer, PP, End Opening, Natural, *Optional, if using to store tissue slides after coverslipping*	Fisher Scientific	HS15986
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
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
Additional Materials			
1000-ml Beakers (5)		-	-
Ultrapure/Milli-Q Water, *from Milli-Q Integral Ultrapure Water System or equivalent*		-	-

Tips & Best Practices

TIPS

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

General Reagent Handling

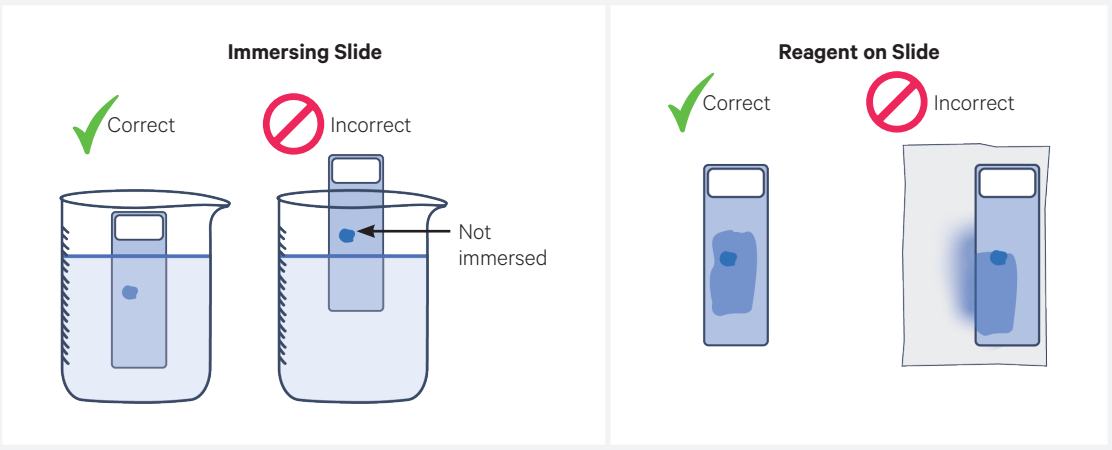
- Thoroughly mix reagents before use.

Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.

Slide Handling

- Always wear gloves when handling slides.
- DO NOT touch the tissue sections on slides.
- Keep tissue slides in a container on dry ice.
- Minimize exposure of slides to sources of particles and fibers.
- When immersing slides in prepared solutions and water, ensure that the tissue sections are completely submerged.
- Keep slides flat on the bench when adding reagents to the tissue.
- Ensure that no absorbent surface is in contact with the reagents on slides during incubation.



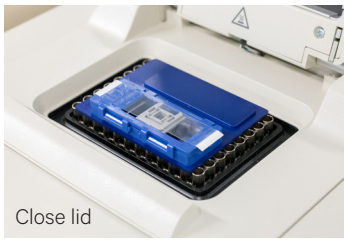
Slide Incubation Guidance

Incubation at a specified temperature

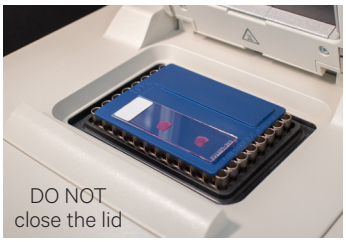
Incubation using a Thermal Cycler:

- 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.

Incubate Assembled Cassette



Incubation on a Thermal Cycler



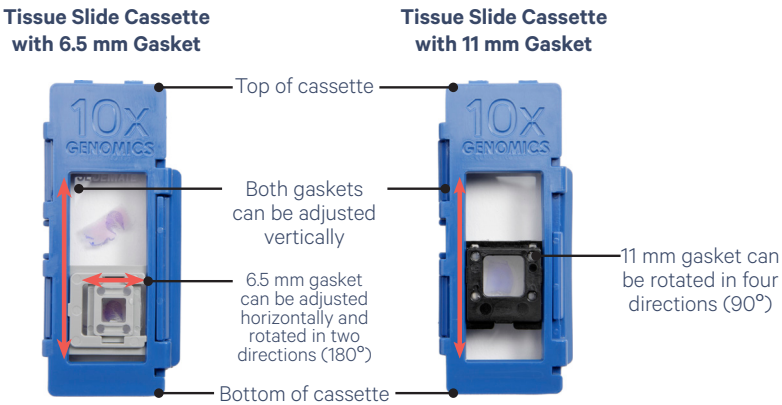
Incubation at room temperature

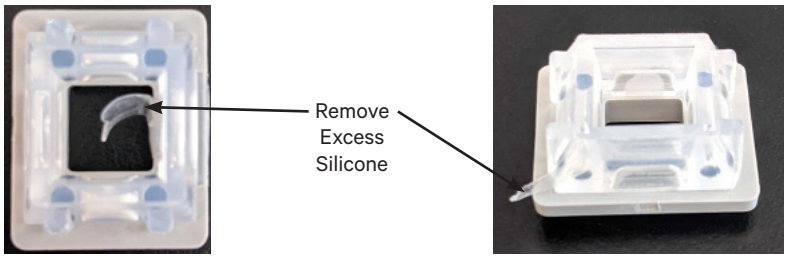
- Place the Visium CytAssist Tissue Slide Cassette on a flat, clean, non-absorbent work surface.

Visium CytAssist Tissue Slide Cassette

- The Visium CytAssist Tissue Slide Cassette encases the slide and creates a leakproof well for adding reagents on tissue slides.
- 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.
- Refer to 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).
- Place slides in the cassette only when specified.



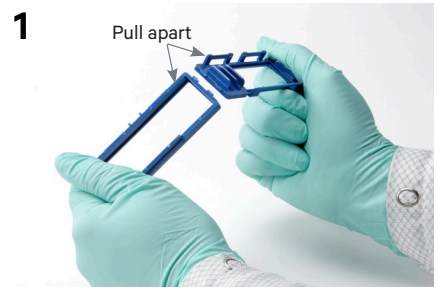


Visium CytAssist Tissue Slide Cassette Assembly

Wear fresh gloves while assembling Tissue Slide Cassette.

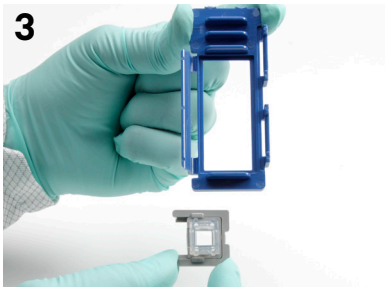
Exercise caution when handling slide edges to prevent injury.

Break cassette into two halves by bending each half at the hinge until they snap apart



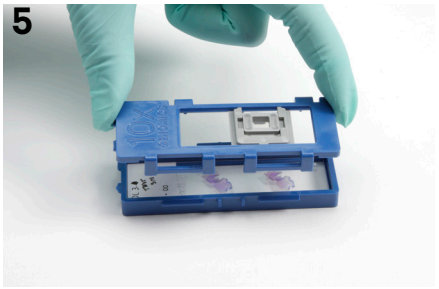
Place tissue slide into lower half of cassette with tissue facing up

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.

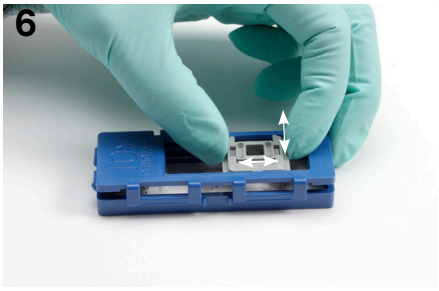


Securely combine gasket with top half of cassette until the gasket snaps into place.

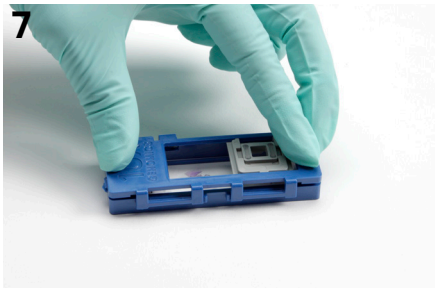
Gently place top half of cassette over bottom half. DO NOT assemble together until Step 7.



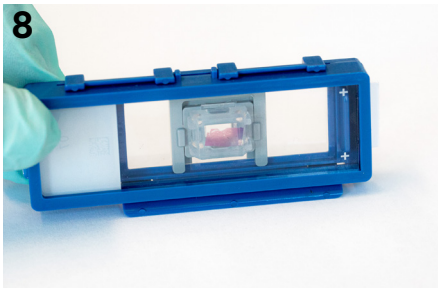
Adjust gasket such that gasket is over the tissue area of interest. The 6.5 mm gasket can be adjusted horizontally as well as vertically.



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

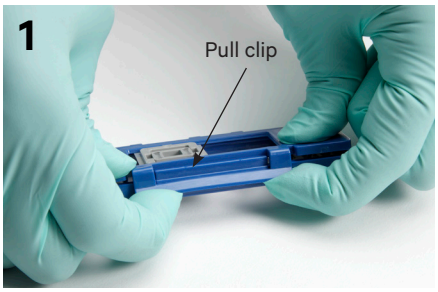


Turn cassette over and verify tissue area of interest is within gasket. DO NOT move gasket once cassette is closed. If necessary, open cassette and recenter gasket.



Visium CytAssist Tissue Slide Cassette Removal

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

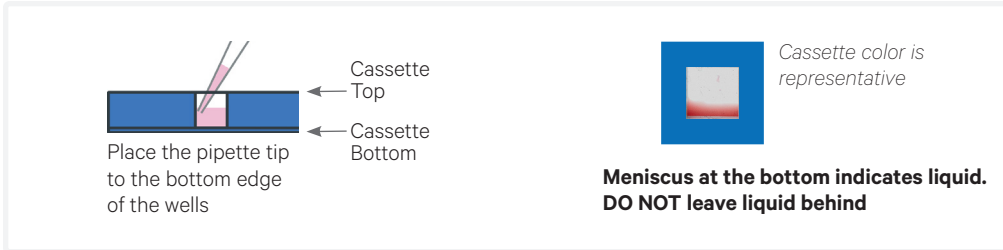
Hold slide by the label and lift slide out from lower half

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

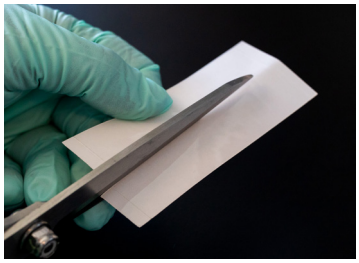
Application

- If applying a Visium Slide Seal to a Tissue Slide Cassette, the seal must be cut in half lengthwise.
- Four pre-cut seals per tissue section are needed for the entire Visium CytAssist Spatial Gene Expression assay.
- 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.

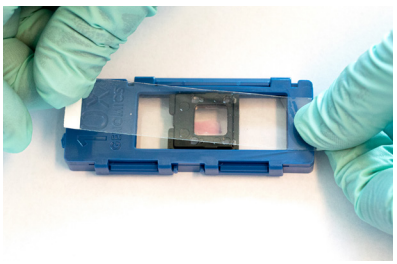
Removal

- 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.

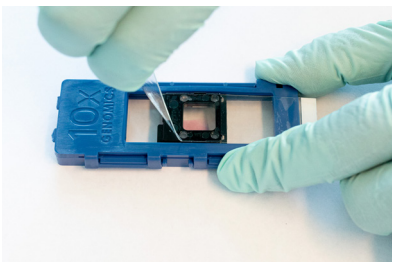
Cut Slide Seal in Half Lengthwise



Visium Slide Seal Application



Visium Slide Seal Removal



Visium CytAssist Tested Slides

The following slides have been tested for use with the Visium CytAssist Tissue Slide Cassette and instrument.

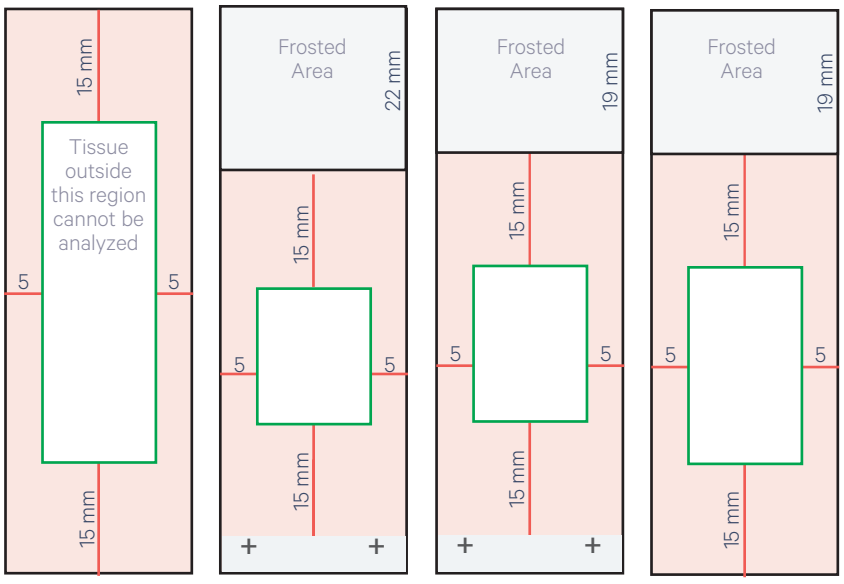
Item	Length (mm)	Width (mm)	Thickness (mm)
Epredia Shandon ColorFrost Plus	75.0	25.0	1.0
Fisherbrand SuperFrost Plus	75.0	25.0	1.0
Sigma-Aldrich Poly Prep Slides	75.0	25.0	1.0
VWR SuperFrost Plus Micro Slide, Premium	75.0	25.0	1.0

If unsure of slide part number, refer to 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



Generic

Epredia Shandon ColorFrost Plus

Fisherbrand SuperFrost Plus

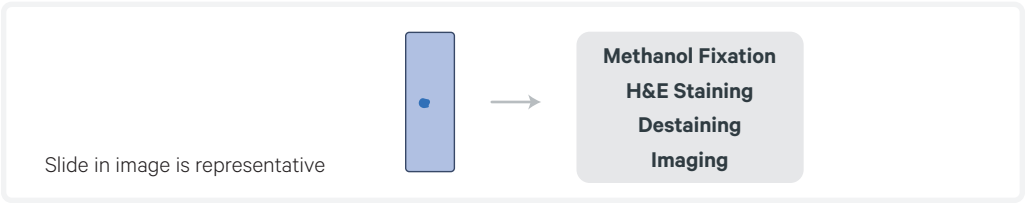
VWR SuperFrost Plus

Sigma-Aldrich Poly Prep

1. Methanol Fixation & H&E Staining

1.0 Overview

This chapter provides guidance on the methanol fixation and H&E staining of glass slides containing fresh frozen tissue sections. Ensure that microscope settings have been verified and imaging programs have been created prior to starting this protocol. Consult the Visium CytAssist Spatial Gene Expression Imaging Guidelines Technical Note (CG000521) for more information.

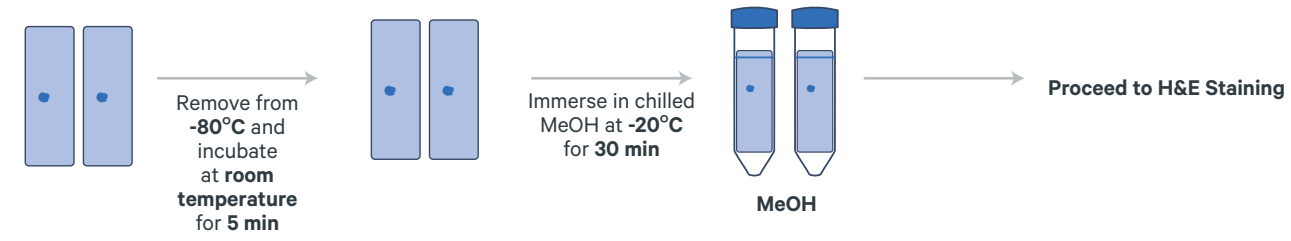


1.1 Preparation - Buffers

For Methanol Fixation	
Prepare fresh, process two slides per jar (slides should face away from each other) unless otherwise indicated.	
Items	**Preparation & Handling**
☐ Methanol	Dispense 30 ml of methanol into a 50-ml centrifuge tube for each slide. Chill methanol to -20°C before use. *Alternatively, use a slide mailer. Each slide mailer may be used for one slide.*
For H&E Staining	
Items	**Preparation & Handling**
☐ Milli-Q or UltraPure Water	Dispense 30 ml of water into a 50-ml centrifuge tube for each slide. Label five 1000-ml beakers as Water Beakers 1 – 5. Dispense 800 ml of water into each beaker. Dispensed volumes in each beaker can be used for two slides. *Alternatively, use 50-ml centrifuge tubes instead of beakers. Each tube may be used for one slide.*
☐ Alcoholic Eosin	30 ml in a 50-ml conical tube. Each tube may be used for one slide.

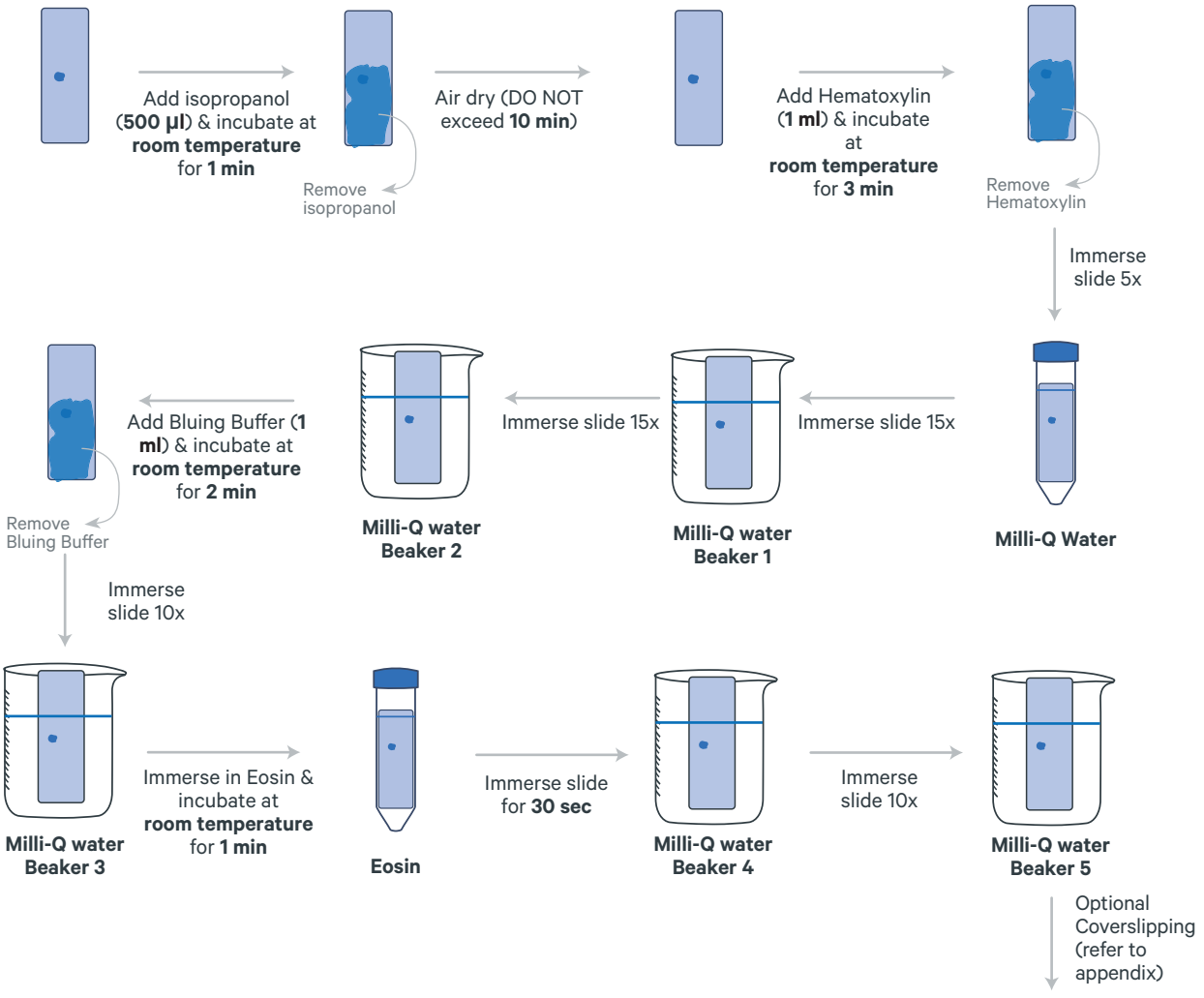
Protocol Overview

Methanol Fixation 35 min



Slide in image is representative

H&E Staining 15 min



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

1.2 Methanol Fixation

a. Retrieve slide from **-80°C** and place on dry ice in a sealed container.

b. Place slide on a flat, clean, non-absorbent work surface for **5 min** at **room temperature**.

c. Completely immerse slide in chilled methanol.

d. Incubate upright for **30 min** at **-20°C**.

1.3 H&E Staining

a. Remove slide from methanol and wipe excess liquid from the back of the slide, without touching the tissue sections. Place on a flat, clean, nonabsorbent work surface. Some residual droplets may remain.

b. Add **500 µl** isopropanol to uniformly cover the entire tissue section on the slide.

c. Incubate **1 min** at room temperature.
When incubating the slide with reagents, ensure that the slide is not in contact with any absorbent surface, like laboratory wipes, which may absorb the reagents.

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

e. Wipe excess liquid from the back of the slide, without touching the tissue sections. Place on a flat, clean, nonabsorbent work surface.

f. Air dry the slide. To prevent tissue section from over drying, inspect slide after **3 min**. DO NOT exceed **10 min**.

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

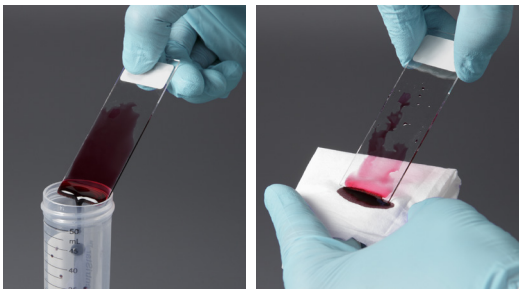
h. Incubate **3 min** at room temperature.

i. 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.

1.3 H&E Staining *contd.*

j. Immerse slide 5x in Milli-Q Water centrifuge tube.

k. Immerse slide 15x in Milli-Q Water Beaker 1.

l. Immerse slide 15x in Milli-Q Water Beaker 2.

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

n. Add **1 ml** Bluing Buffer to uniformly cover all tissue sections.

o. Incubate **2 min** at **room temperature**.

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

q. Immerse slide 10x in Milli-Q Water Beaker 3.

r. Immerse slide in the Alcoholic Eosin centrifuge tube.

s. Incubate **1 min** at room temperature.

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

u. Immerse slide for **30 sec** in Milli-Q Water Beaker 4.

v. Immerse slide 10x in Milli-Q Water Beaker 5.

w. Wipe the back of the slide with a laboratory wipe. Place on a flat, clean, nonabsorbent work surface and air dry until tissue is opaque.

x. Proceed to Tissue Imaging.

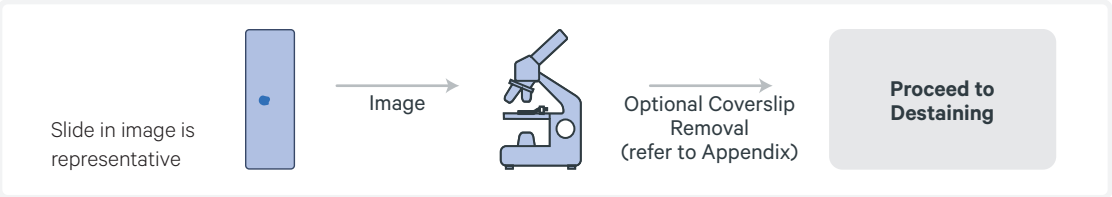
STOP

Optional: A coverslip may be mounted on the slide prior to imaging or for storage. See Appendix for Coverslip Application & Removal protocol and stopping point guidance.

2. Tissue Imaging

2.0 Overview

This chapter provides guidance on imaging tissue slides containing H&E stained fresh frozen sections.



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 slide individually at the desired magnification using brightfield imaging settings.

b. Consult the Visium CytAssist Spatial Gene Expression Imaging Guidelines Technical Note (CG000521) for additional information.

c. After imaging, proceed **immediately** to Destaining for H&E Sections.

3. Destaining for H&E Stained Sections

3.0 Overview

This chapter provides guidance on performing destaining. If coverslip was mounted on the tissue slide, ensure that the coverslip is removed prior to destaining.

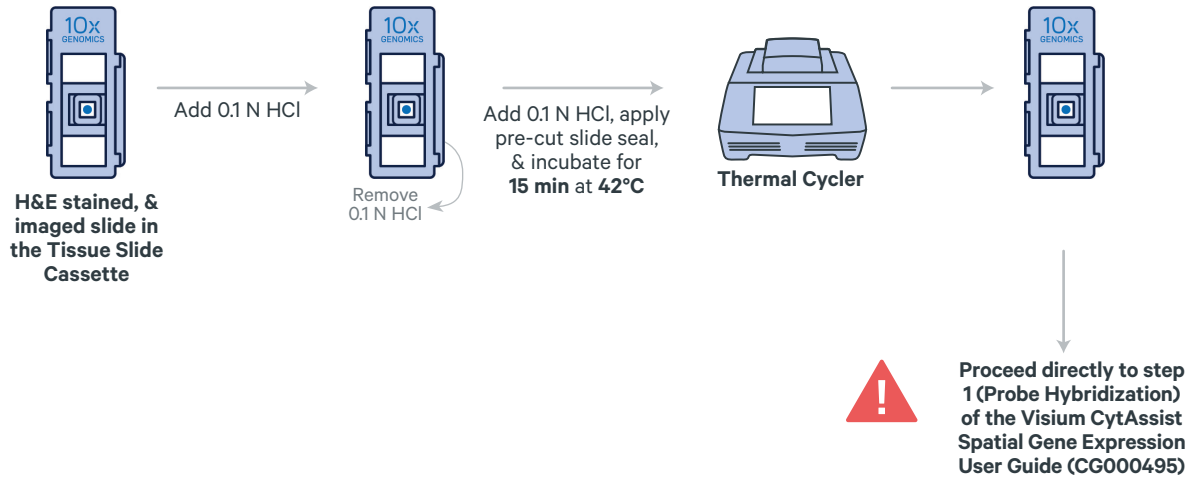
Once sections have been destained, step 1 (Probe Hybridization) of Visium CytAssist Spatial Gene Expression User Guide (CG000495) should be immediately performed.

3.1 Preparation - Buffers

For Destaining	
Items	**Preparation & Handling**
☐ 0.1 N HCl	If necessary, prepare 0.1N HCl using nuclease-free water.

Protocol Overview

Destaining ~15 min



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.

TIPS

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 without introducing bubbles. Tap cassette gently to ensure uniform coverage.

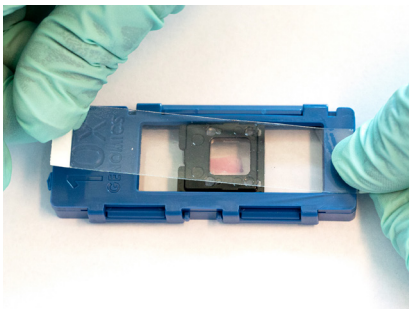
d. Remove HCl from the wells.

e. Add ■**100 µl or** ▲**200 µl** 0.1 N HCl along the side of the wells 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**.

Add Reagent

Apply Visium Slide Seal

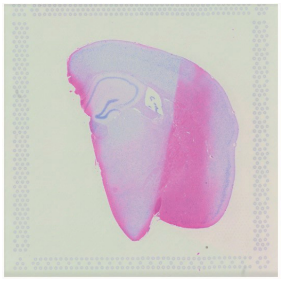
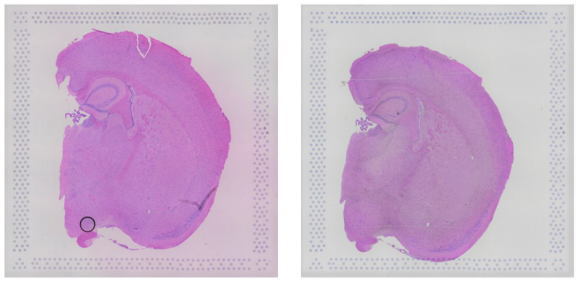


g. Close the thermal cycler lid. Skip Pre-equilibrate step to initiate Destaining.

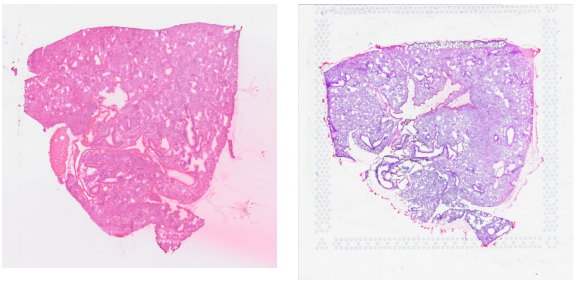
h. Remove the cassette from the Low Profile Thermocycler Adapter and place on a flat, clean work surface. Some color remaining in the tissue after Destaining is normal.

i. Proceed **immediately** to step 1 (Probe Hybridization) of the Visium CytAssist Spatial Gene Expression User Guide (CG000495) Rev C or later.

Troubleshooting

	Notes
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 by using a wide-bore pipette tip.  **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.  Slide in image is representative
Incorrect Staining Protocol	• Ensure that the correct staining protocol with fresh reagents was followed.  **Incorrect staining protocol (right image) may result in poor staining performance.** Slide in image is representative

Troubleshooting

	Notes
Incorrect Eosin	• Ensure that the correct eosin (alcoholic) is used for staining.  **Alcoholic eosin (left) stains optimally as compared to aqueous eosin (right).**

Appendix

Coverslip Application

A coverslip may be mounted on tissue slides before imaging to enhance optical quality. Although imaging without a coverslip is sufficient to visualize the tissue morphology, some imaging systems or higher imaging magnifications require coverslips.

If using a coverslip, use the following protocol to ensure that the tissue sections are not damaged. Coverslipped tissue slides can be stored prior to or after imaging.

Buffer Preparation

Items	Preparation & Handling
☐ Mounting Medium	The dilution below is not necessary if stock glycerol is already at 85%. Invert or pipette gently with a wide-bore pipette tip to mix. Briefly centrifuge to remove bubbles.

Mounting Medium	Stock	Final	1X (µl)	2X+ 15% (µl)
Glycerol	100%	85%	85.0	195.5
Nuclease-free Water	100%	15%	15.0	34.5
Total	**-**	**-**	**100.0**	**230.0**

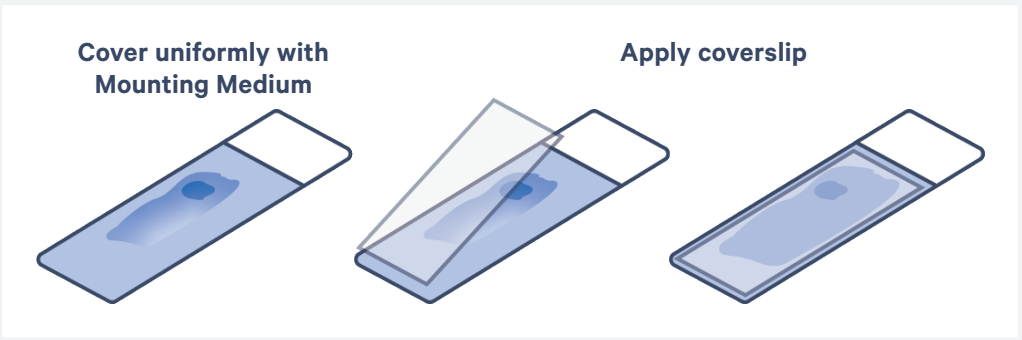
DO NOT let the attached coverslip dry.
DO NOT use Cytoseal or nail polish for securing the coverslip.

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

b. Using a **wide-bore** pipette tip, add **100 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.



STOP

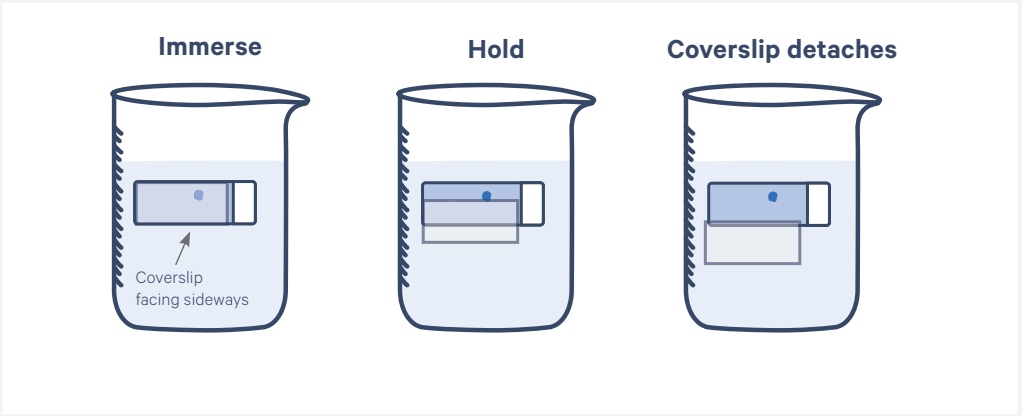
e. Once coverslipping is complete, proceed with imaging or store slides in a container with tissue facing upward at **4°C** in the dark for up to **1 week**. Slides should not be coverslipped for more than **1 week** total before being destained.

Coverslip Removal

a. Dispense **800 ml** Milli-Q water in a beaker. Up to 8 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.

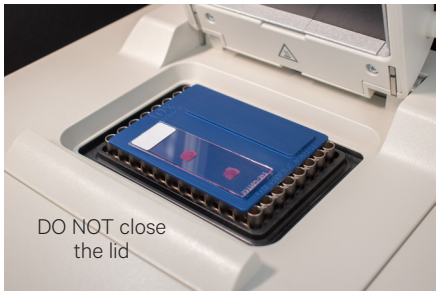


To avoid damaging the tissue sections or causing tissue detachment, DO NOT move the slide up and down, shake forcibly or manually move the coverslip.

d. Gently immerse slides 30x in water to ensure all Mounting Medium is removed.

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

f. Incubate slides on the Low Profile Thermocycler Adapter with the thermal cycler lid open for **3 min** at **37°C** to dry the tissue. Ensure tissue is completely dry.



STOP

g. Proceed **immediately** to step 3.2 Destaining or store slides vertically in a slide mailer or 50-ml conical at **4°C** in the dark for up to **24 h**. Slides can only be stored after coverslip removal if they have not previously been stored for 1 week after coverslipping. During storage, ensure slides do not touch one another.

Document Revision Summary

Document Number	CG000614
Title	Visium CytAssist Spatial Gene Expression for FF – Methanol Fixation, H&E Staining, Imaging & Destaining
Revision	Rev A
Revision Date	January 2023

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