

PLANNER

Visium Spatial Gene Expression Protocol Planner

This document provides the time planners and additional equipment, kits, and reagents for the Visium Spatial Gene Expression and Tissue Optimization protocols. The 10x Genomics Visium Spatial Reagent Kits are not listed in this document.

H&E Stained Sections

An overview of protocol steps and necessary reagents for performing the Visium Spatial Gene Expression and Tissue Optimization workflows with H&E stained tissues is provided below.

H&E Staining - Visium Spatial Tissue Optimization Protocol Steps & Timing

4 h



Steps	Timing
Tissue Staining & Imaging	
Methanol Fixation, H&E Staining, & Imaging Demonstrated Protocol (CG000160)	
Tissue Fixation	35 min
Tissue Staining	30 min
Tissue Imaging*	Variable
Step 1 – Permeabilization & cDNA Synthesis	
Visium Spatial Tissue Optimization Reagent Kits User Guide (CG000238)	
1.1 Tissue Permeabilization	Variable
1.2 Fluorescent cDNA Synthesis	60 min
Step 2 – Tissue Removal	
Visium Spatial Tissue Optimization Reagent Kits User Guide (CG000238)	
2.1 Tissue Removal	70 min
2.2 Slide Imaging*	Variable

*~4 h workflow, excluding imaging steps

H&E Staining - Visium Spatial Gene Expression Protocol Steps & Timing

1-1.5 days



Steps	Timing	Stop & Store
Tissue Staining & Imaging		
Methanol Fixation, H&E Staining, & Imaging Demonstrated Protocol (CG000160)		
Tissue Fixation	35 min	
Tissue Staining	30 min	
Tissue Imaging	Variable	
Step 1 – cDNA Synthesis		
Visium Spatial Gene Expression Reagent Kits User Guide (CG000239)		
1.1 Tissue Permeabilization	Variable	
1.2 Reverse Transcription	65 min	
Step 2 – Second Strand Synthesis & Denaturation		
Visium Spatial Gene Expression Reagent Kits User Guide (CG000239)		
2.1 Second Strand Synthesis	25 min	
2.2 cDNA Denaturation	15 min	
Step 3 – cDNA Amplification & QC		
Visium Spatial Gene Expression Reagent Kits User Guide (CG000239)		
3.1 Cycle Number Determination – qPCR	45 min	
3.2 cDNA Amplification	45-60 min	4°C ≤72 h or -20°C ≤1 week
3.3 cDNA Cleanup – SPRIselect	20 min	4°C ≤72 h -20°C ≤4 weeks
3.4 cDNA QC & Quantification	50 min	
Step 4 – Visium Spatial Gene Expression Library Construction		
Visium Spatial Gene Expression Reagent Kits User Guide (CG000239)		
4.1 Fragmentation, End Repair & A-tailing	50 min	
4.2 Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	30 min	
4.3 Adaptor Ligation	25 min	
4.4 Post Ligation Cleanup- SPRIselect	20 min	
4.5 Sample Index PCR	40 min	4°C ≤72 h
4.6 Post Sample Index PCR Double Sided Size Selection- SPRIselect	30 min	4°C ≤72 h or -20°C long term
4.7 Post Library Construction QC	50 min	

Only required for Methanol Fixation, H&E Staining & Imaging for Visium Spatial Protocols Demonstrated Protocol (CG000160)

The items in the table below have been validated by 10x Genomics and are highly recommended for the Visium Spatial Reagent Kits protocols. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment such as water baths, centrifuges, vortex mixers, pH meters, freezers etc.

Tissue Fixation		
Vendor	Item	Part Number
Millipore Sigma	Methanol, for HPLC, ≥99.9%	34860
Tissue H&E Staining		
Vendor	Item	Part Number
Millipore Sigma	Acetic Acid, ≥99.9%	A6283
	2-Propanol (Isopropanol), ≥99.5%	I9516-25ML
	Eosin Y solution, aqueous	HT110216-500ML
	Eosin Y-solution, 0.5% aqueous (alternative to HT110216-500ML)	1098441000
	Hematoxylin Solution, Mayer's (alternative to Agilent product)	MHS16-500ML
	Hematoxylin solution according to Mayer (alternative to Agilent product)	51275-100ML
	Protector RNase Inhibitor	3335399001
Agilent	Hematoxylin, Mayer's (Lillie's Modification)	S30930-2
	Bluing Buffer, Dako	CS70230-2
	Eosin, Dako	CS70130-2
	(alternative to Millipore Sigma product)	
Thermo Fisher Scientific	Tris Base (White Crystals or Crystalline Powder/Molecular Biology)	BP152-500
	Shandon Bluing Reagent (alternative to Agilent product)	6769001
	RiboLock RNase Inhibitor (Optional - alternative to Millipore Sigma product)	E00382
Corning	Corning 250 mL Vacuum System, 0.2 µm Pore 19.6cm ² NY Membrane	430771
	Self-Standing Polypropylene Centrifuge Tubes, 50 ml, sterile	430921
Additional Materials		
-	Dry Ice	-
-	Ultrapure/Milli-Q water (from Milli-Q Integral Ultrapure Water System or equivalent)	-

Immunofluorescence Stained Sections An overview of protocol steps and necessary reagents for performing the Visium Spatial Gene Expression and Tissue Optimization workflows with immunofluorescence stained tissues is provided below.

Immunofluorescence Staining - Visium Spatial Tissue Optimization Protocol Steps & Timing




Steps	Timing
Tissue Staining & Imaging	
Methanol Fixation, H&E Staining, & Imaging Demonstrated Protocol (CG000160)	
Tissue Fixation	35 min
Primary Antibody Staining	45 min
Secondary Antibody Staining†	45 min
Tissue Imaging	Variable
Step 1 – Permeabilization & cDNA Synthesis	
Visium Spatial Tissue Optimization Reagent Kits User Guide (CG000238)	
1.1 Tissue Permeabilization	Variable
1.2 Fluorescent cDNA Synthesis	60 min
Step 2 – Tissue Removal	
Visium Spatial Tissue Optimization Reagent Kits User Guide (CG000238)	
2.1 Tissue Removal	70 min
2.2 Slide Imaging*	Variable

*~4 h workflow, excluding imaging steps

† Omit if using fluorophore conjugated primary antibodies.

Immunofluorescence Staining - Visium Spatial Gene Expression Protocol Steps & Timing

1-1.5 days

Steps		Timing	Stop & Store
Tissue Staining & Imaging			
Methanol Fixation, Immunofluorescence Staining, & Imaging Demonstrated Protocol (CG000160)			
	Tissue Fixation	35 min	
	Primary Antibody Staining	45 min	
	Secondary Antibody Staining*	45 min	
	Tissue Imaging	Variable	
Step 1 – cDNA Synthesis			
Visium Spatial Gene Expression Reagent Kits User Guide (CG000239)			
1.1	Tissue Permeabilization	Variable	
1.2	Reverse Transcription	65 min	
Step 2 – Second Strand Synthesis & Denaturation			
Visium Spatial Gene Expression Reagent Kits User Guide (CG000239)			
2.1	Second Strand Synthesis	25 min	
2.2	cDNA Denaturation	15 min	
Step 3 – cDNA Amplification & QC			
Visium Spatial Gene Expression Reagent Kits User Guide (CG000239)			
3.1	Cycle Number Determination – qPCR	45 min	
3.2	cDNA Amplification	45-60 min	 4°C ≤72 h or -20°C ≤1 week
3.3	cDNA Cleanup – SPRIselect	20 min	 4°C ≤72 h -20°C ≤4 weeks
3.4	cDNA QC & Quantification	50 min	
Step 4 – Visium Spatial Gene Expression Library Construction			
Visium Spatial Gene Expression Reagent Kits User Guide (CG000239)			
4.1	Fragmentation, End Repair & A-tailing	50 min	
4.2	Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	30 min	
4.3	Adaptor Ligation	25 min	
4.4	Post Ligation Cleanup- SPRIselect	20 min	
4.5	Sample Index PCR	40 min	 4°C ≤72 h
4.6	Post Sample Index PCR Double Sided Size Selection- SPRIselect	30 min	 4°C ≤72 h or -20°C long term
4.7	Post Library Construction QC	50 min	

*Omit if using fluorophore conjugated primary antibodies.

Only required for Methanol Fixation, Immunofluorescence Staining & Imaging for Visium Spatial Protocols Demonstrated Protocol (CG000312)

The items in the table below have been validated by 10x Genomics and are highly recommended for the Visium Spatial Reagent Kits protocols. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment such as water baths, centrifuges, vortex mixers, pH meters, freezers etc.

Tissue Fixation		
Vendor	Item	Part Number
Millipore Sigma	Methanol, for HPLC, ≥99.9%	34860
Tissue IF Staining		
Vendor	Item	Part Number
Corning	Self-Standing Polypropylene Centrifuge Tubes, 50 ml, sterile	430921
Sigma Aldrich	Triton X-100	93443-100ML
Thermo Fisher Scientific	Thermo Scientific Signature Series Cover Glasses	22-050-233
	Shandon ColorFrost Plus Slides (Alternatively, use any 75 x 25 x 1 mm slide)	6776214
	Fisherbrand Superfrost Plus Microscope Slides (Optional - Alternatively, use any 75 x 25 x 1 mm slide)	12-550-15
	DAPI Solution	62248
	RiboLock RNase Inhibitor (Alternative to Millipore Sigma product)	E00382
Millipore Sigma	SSC Buffer 20x Concentration	S6639L
	Protector RNase Inhibitor	3335402001
Miltenyi Biotec	MACS BSA Stock Solution	130-091-376
BioLegend	Human TruStain FcX (Fc Receptor Blocking Solution) (Alternatively, use any species appropriate Fc blocking solution)	422301
	TruStain FcX (anti-mouse CD16/32) Antibody	101309
New England Biolabs	Ribonucleoside Vanadyl Complex	S1402S
	RNase Inhibitor, Murine (Alternatively to Millipore Sigma product)	M0314L
Invitrogen	Alexa Fluor 488 Phalloidin (Optional)	A12379
	Alexa Fluor 594 Phalloidin (Optional)	A12381
	Alexa Fluor 647 Phalloidin (Optional)	A22287
-	Primary Antibodies	-
-	Secondary Antibodies	-
Additional Materials		
-	Dry Ice	-
-	Glycerol	-
-	Isopropanol	-
-	Ultrapure/Milli-Q water (from Milli-Q Integral Ultrapure Water System or equivalent)	-
-	Forceps	-

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Visium Spatial Reagent Kits protocols. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment such as water baths, centrifuges, vortex mixers, pH meters, freezers etc.

Supplier	Description	Part Number (US)
Plastics		
Eppendorf	PCR Tubes 0.2 ml 8-tube strips	951010022
	DNA LoBind Tubes, 1.5 ml	022431021
	DNA LoBind Tubes, 2.0 ml (when processing more than 2 slides)	022431048
USA Scientific	TempAssure PCR 8-tube strip	1402-4700
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml	N8010580
	MicroAmp 8 -Cap Strip, clear	N8010535
	Simport Scientific LockMailer Tamper Evidence Slide Mailer (alternatively, use a 50-ml centrifuge tube)	22-038-399
Corning	Self-Standing Polypropylene Centrifuge Tubes (50 ml), sterile	430921
Bio-Rad	Hard-shell PCR Plates 96-well, thin wall (pkg of 50) (alternatively, use any compatible PCR Plate)	HSP9665
	Microseal 'B' PCR Plate Sealing Film, adhesive (alternatively, use any PCR Plate sealing adhesive)	MSB1001
Rainin	Tips LTS 200UL Filter RT-L200FLR	30389240
	Tips LTS 1ML Filter RT-L1000FLR	30389213
	Tips LTS 20UL Filter RT-L10FLR	30389226
VWR	Divided Polystyrene Reservoirs	41428-958
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water	AM9937
	Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	12090-015
	Tris 1M, pH 7.0, RNase-free	AM9850G
	Universal Mouse Reference RNA* (Optional. Alternatively, use any bulk Total RNA. 1µg/µl, RIN ≥ 7)	QS0640
Fisher Chemical	Hydrochloric Acid Solution, 0.1N	SA54-1
KAPA Biosystems	KAPA SYBR FAST qPCR Master Mix (2X)	KK4600
Beckman Coulter	SPRIselect Reagent Kit	B23318

* Only required for Visium Spatial Tissue Optimization protocol

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Visium Spatial Reagent Kits protocols. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment such as water baths, centrifuges, vortex mixers, pH meters, freezers etc.

Supplier	Description	Part Number (US)
Kits & Reagents		
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous) Potassium Hydroxide Solution, 8M SSC Buffer 20X Concentrate Sodium dodecyl sulfate (SDS) solution, 10% in water*	E7023-500ML P4494-50ML S66391L 71736
Qiagen	Qiagen Buffer EB	19086
-	Ultrapure/Milli-Q water (from Milli-Q Integral Ultrapure Water System or equivalent)	
Equipment		
Labnet	Slide Spinner* (alternatively, use a 50-ml centrifuge tube in a centrifuge with a swing-bucket)	C1303-T
Rainin	Pipet-Lite Multi Pipette L8-200XLS+ Pipet-Lite LTS Pipette L-2XLS+ Pipet-Lite LTS Pipette L-10XLS+ Pipet-Lite LTS Pipette L-20XLS+ Pipet-Lite LTS Pipette L-100XLS+ Pipet-Lite LTS Pipette L-200XLS+ Pipet-Lite LTS Pipette L-1000XLS+	17013805 17014393 17014388 17014392 17014384 17014391 17014382
VWR	VWR Mini Centrifuge (alternatively, use any equivalent mini centrifuge)	76269-064
Quantification & Quality Control		
Agilent	2100 Bioanalyzer Laptop Bundle High Sensitivity DNA Kit 4200 TapeStation High Sensitivity D1000 ScreenTape/Reagents High Sensitivity D5000 ScreenTape/Reagents	G2943CA 5067-4626 G2991AA 5067-5592/ 5067-5593 5067-5584/ 5067-5585
PerkinElmer	LabChip GX Touch HT Nucleic Acid Analyzer DNA High Sensitivity Reagent Kit	CLS137031 CLS760672
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms	KK4824

* Only required for Visium Spatial Tissue Optimization protocol

Cryostat Specifications

The Cryostar NX70 Cryostat with listed features was used by 10x Genomics. Any equivalent system with the listed features may be used.

Component	Features
Main Cryochamber	Maintains stable temperatures from –10°C to –20°C
Cryostat Blade	Separate and adjustable temperature control Maintains stable temperatures from –35°C to –5°C
Specimen Head	Separate and adjustable temperature control Maintains stable temperatures from –50°C to +10°C X-axis and Y-axis adjustment
Blade Holder Base	Adjustable cutting angle Adjustable blade position Section thickness 10-50 µM
Cryobar	Rapid cooling

Additional Items for Cryosectioning

Vendor	Item	Part Number
VWR	TissueTek O.C.T. Compound	25608-930
	Sterile Centrifuge Tubes with Flat Caps, 50 ml	82018-050
10x Genomics	Visium Spatial Tissue Optimization Slide/ Visium Spatial Gene Expression Slide/ Visium Gateway Gene Expression Slide	3000394/ 2000233/ 2000363
Thermo Fisher Scientific	CryoStar NX70 Cryostat Vacutome, Low Profile Blade Carrier	957020
	Shandon ColorFrost Plus Slides 75 x 25 x 1 mm (Alternatively, use any 75 x 25 x 1 mm slide)	6776214
	Fisherbrand Superfrost Plus Microscope Slides (Optional - alternatively, use any 75 x 25 x 1 mm slide)	12-550-15
	Flat cryostat brush, 10 mm	334160
	Brush, small beveled	334171
	Magnetic Brush, big	334172
Fisher Scientific	Thermo Scientific CryoStar NX70 Specimen Chuck	14-071-413
	Simport Scientific LockMailer Tamper Evident Slide Mailer (Alternatively, use a 50-ml centrifuge tube)	22-038-399
	MX35 Ultra Microtome Blade Low Profile	30-538-35350
	Glass Anti-Roll Plate	A78930200
Additional Materials		
-	Razor Blades	-
-	Dry Ice	-
-	Tissue Forceps	-

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

Recommended Real Time qPCR Systems

Supplier	Description	Part Number
Applied Biosystems	QuantStudio 12K Flex system	4471087
Bio-Rad	CFX96 Real-time System	1855196

For complete protocol information, consult the Visium Spatial Tissue Optimization Reagent Kits User Guide (CG000238) and the Visium Spatial Gene Expression Reagent Kits User Guide (CG000239).

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