

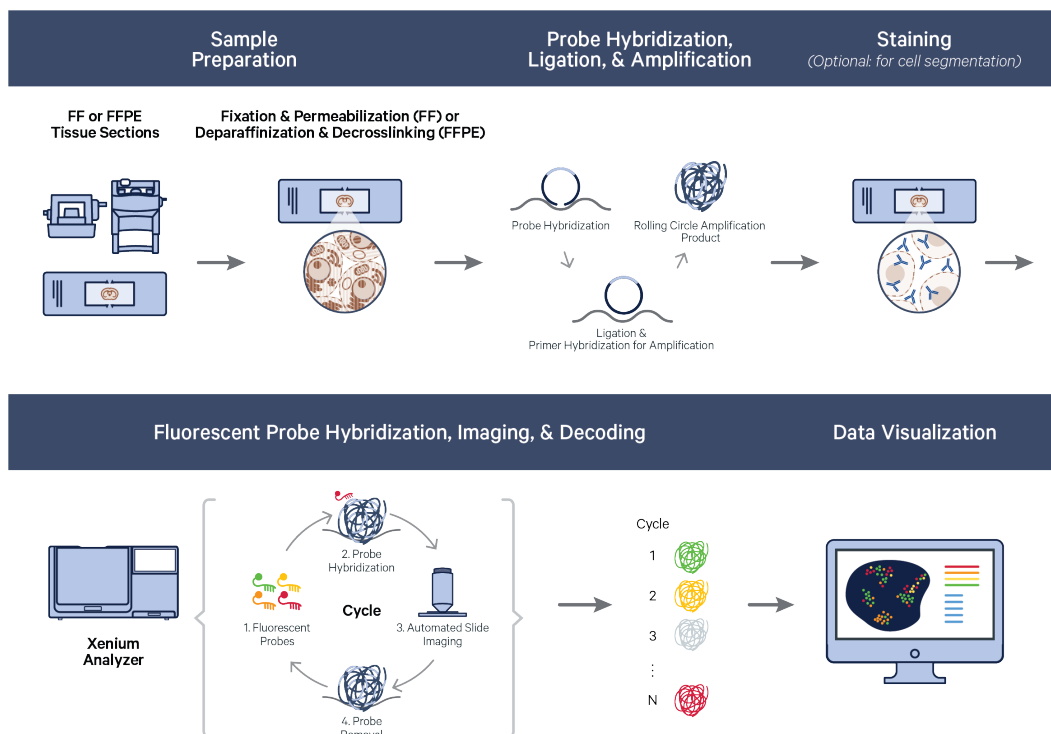
Xenium In Situ Gene Expression – Protocol Planner

Introduction

Xenium In Situ measures gene expression in tissue sections derived from either formalin fixed and paraffin embedded (FFPE) or fresh frozen (FF) tissue samples placed on Xenium Slides. The Xenium In Situ Gene Expression workflow can be paired with optional cell segmentation staining. This Protocol Planner provides an overview of the workflow along with the Xenium Analyzer overview. To enable efficient planning, a breakdown of key protocol steps and times, list of user-acquired reagents and consumables, and information about supporting documentation that will be available for executing the Xenium workflows is also provided.

10x Genomics Xenium Reagent Kits are not listed in this document.

Xenium In Situ Gene Expression Workflow Overview



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Introduction

The protocol planner provides resources and guidelines to prepare a laboratory for seamless planning and execution of the Xenium In Situ Gene Expression workflow. The key topics covered in this document are highlighted below.

Xenium Analyzer Overview

The document provides a high level overview of key instrument dimensions, delivery, installation, training along with guidelines for site preparation.

Workflow Documents

A list of documents to support various steps of the in situ workflow is provided for formalin-fixed & paraffin-embedded (FFPE) and fresh frozen (FF) and tissue samples. These documents, along with many additional resources, will be available on the 10x Genomics Support website once the Xenium Analyzer is installed and ready to use.

Key Protocol Steps & Timing

A breakdown of the off-instrument and on-instrument workflow steps, the time required to perform each step, and safe stopping points are provided.

Reagents & Consumables

(not supplied by 10x Genomics)

The reagents & consumables for various steps of the Xenium In Situ workflow are listed in this document. The Appendix also includes a list of items for optional H&E staining along with post-run quencher removal (only if staining slides after the instrument run). The listed items have been tested by 10x and perform optimally with the assay. These items will not be supplied by 10x Genomics and should be acquired from the indicated vendors. Refer to the manufacturer's website for regional part numbers. For items with multiple options, choose one based on availability and preference.

Substituting materials may adversely affect system performance. This list may not include some standard laboratory equipment.



Some reagents and consumables, such as PBS, Tween, ice buckets etc., are common across multiple steps of the workflow and need not be bought individually for each step.

Reagents & Consumables *(not supplied by 10x Genomics)*

Approximate volumes of bulk (≥ 100 ml) reagents used for preparing and processing samples on two Xenium Slides per Xenium instrument run are listed below.



For precise volumes of all reagents, consult relevant workflow protocols.

Volume of Bulk Reagents (≥ 100 ml)			
Item	~Volumes for two Xenium Slides (ml)		
	Sample Prep + Probe Hyb.	Instrument Run Only	Total
Nuclease-free Water	110	1,140*	1,250
Ultrapure Water/Milli-Q Water	-	1,000	1,000
10X PBS	10	100	110
100% DMSO	-	150	150
Xylene <i>(only for FFPE samples)</i>	100	-	100
Ethanol <i>(only for FFPE samples)</i>	285	-	285

*For instrument run ONLY: nuclease-free water can be substituted with nuclease-free Ultrapure/Milli-Q water.



For instrument run during installation and also during training, twice the amount of indicated reagent volumes are needed.

Gene Panel Selection

Prior to executing the Xenium In Situ Gene Expression workflow, ensure that a compatible gene panel has been selected. 10x Genomics provides the option of using pre-designed gene panels. Additionally, the pre-designed panel may be customized by adding genes of interest.

- Visit the 10x Genomics Support website for information regarding all available panels.
- Custom gene panels: Contact 10x Genomics via email at customerservice@10xgenomics.com for information about designing custom gene panels that are compatible with pre-designed panels. The lead time for acquiring custom panels is up to ~8 weeks (1-4 weeks for design, 4 weeks for manufacturing).

Visit the 10x Genomics website for the most current information.

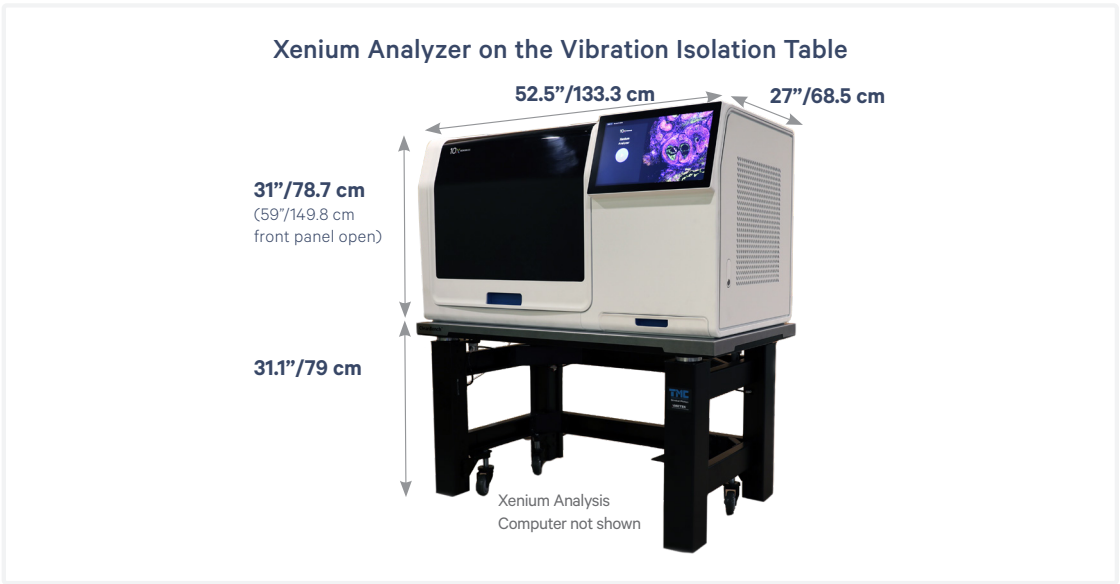
1.0 Xenium Analyzer Overview

Xenium is an end-to-end platform from 10x Genomics that provides highly sensitive, targeted gene expression information at sub-cellular resolution. This platform is powered by the Xenium Analyzer, a versatile instrument for fully automated high-throughput in situ analysis.

1.1 Dimensions

Dimensions	Length	Width	Height
Xenium Analyzer	52.5"/133.3 cm	27"/68.5 cm	31"/ 78.7 cm 59"/149.8 cm - door open
Xenium Analysis Computer	7"/17.8 cm	26.5"/67.3 cm	18"/ 45.7 cm
Vibration Isolation Table	53.2"/135 cm	29.9"/76 cm	31.1"/79 cm
UPS (APC SRT3000XLT* or similar; not provided by 10x Genomics)	3.4"/8.5 cm	25"/63.5 cm	17"/43.2 cm

**Use equivalent regional models*



For detailed specifications, consult the Xenium Analyzer Site Preparation Survey (CG000587). Specifications also be available in the Xenium Analyzer User Guide (CG000584).

1.2 Xenium Analyzer - Installation & Training

An overview of the delivery and installation process is provided below.

1. Site Prep Survey (CG000587)

After an introductory call with 10x Genomics, fill & return the Xenium Analyzer Site Preparation Survey to 10x Genomics

2. Site Readiness Visit

On-site visit by a 10x Genomics Rep. to verify that the site is ready to receive the shipment

3. Shipment

Shipment is received on-site & stored without unboxing until installation

*(*shipped items listed below)*

4. Installation (~5-7 d)

Instrument is installed and verified by a 10x Service Engineer

5. On-site Training (~2-3 d)

On-site training by a 10x FAS

(remote workflow & data analysis trainings will also be provided by 10x and may happen prior to the on-site training; ~2 d)

Ready for Xenium In Situ Gene Expression!

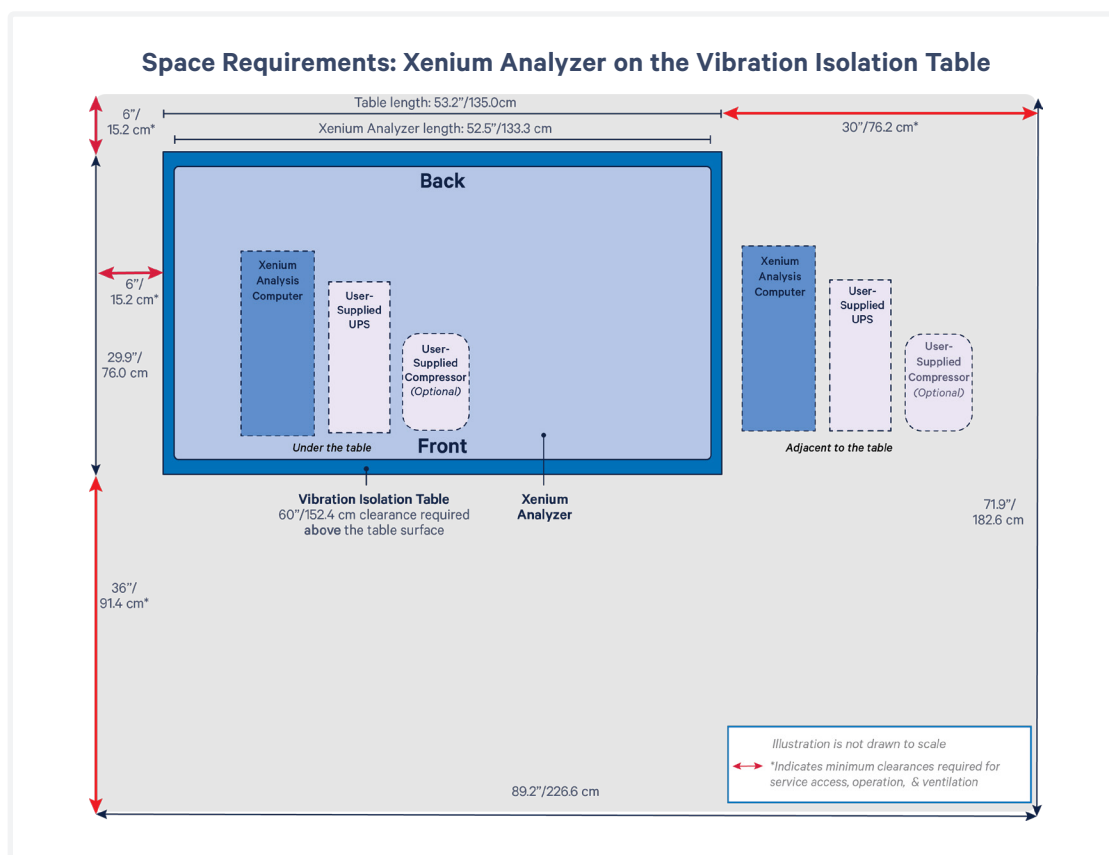
1.3 Xenium Analyzer - Site Preparation

Space Requirements

It is critical to install the instrument in a location away from any vibration sources, such as equipment with compressors (refrigerators, freezers, etc.), motors (centrifuges, shakers, etc.), doors, and busy walkways. Additionally, the installation space should not have soft floor types, such as linoleum or carpet. The instrument should not be placed in direct sunlight or next to other heat generating sources.

The illustration below provides the dimensions and configuration of the space required for installing the Xenium Analyzer. The clearances specified are required for instrument installation, operation, service access, and ventilation. The Xenium Analysis Computer, user-acquired UPS, and compressor (optional) may be placed under the Vibration Isolation Table or adjacent to it with the indicated clearances.

To float the instrument on the Vibration Isolation Table, on-site CDA (compressed dry air) is highly recommended. Alternatively, a low noise/vibration compressor may be used (such as, Air Compressor, Low Noise, 3.5 Liter Capacity, 110 VAC, Model ACPG from Newport or equivalent).



Fill and share the Xenium Analyzer Site Preparation Survey (CG000587) with 10x Genomics, which will be followed by more in-depth discussion with a 10x Representative.

1.3 Xenium Analyzer - Site Preparation *contd.*



Power Supply

The Xenium Analyzer and the Xenium Analysis Computer require uninterrupted power supply for a successful run (~2-4 days/run). Standard emergency generator-backed power is often not uninterruptible and a brief power outage is typical before power resumes. Any interruption in the power supply will terminate the run, resulting in the potential loss of samples, reagents, and data that cannot be replaced/recovered by 10x Genomics.

A user-supplied uninterruptible power supply (UPS) is highly recommended but not required during installation. It is recommended that the instrument should be connected to a UPS during runs (provides ~5 min backup power for 2,000 W). Additionally, connecting the UPS to an emergency generator-backed power supply is recommended.

UPS

The recommended UPS specifications are provided below.

- **Minimum UPS power rating (VA):** 3,000 VA
- **Minimum UPS power rating (W):** 2,700 W
- **Nominal Input Voltage:** 208 V
- **UPS design topology type:** On Line
- **Minimum backup run time for 2,000 W:** ~5 min

APC SRT3000XLT or similar UPS may be used.

Follow the manufacturer's instructions for UPS setup and ensure that the battery is connected.

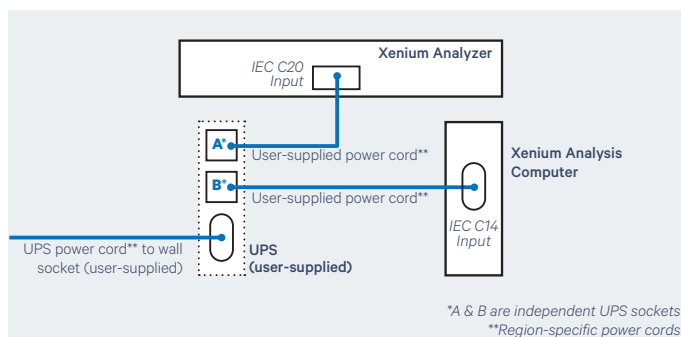
1.3 Xenium Analyzer - Site Preparation *contd.*

The configurations of the Xenium Analyzer and the Xenium Analysis Computer with a UPS (recommended) and without a UPS are illustrated below.

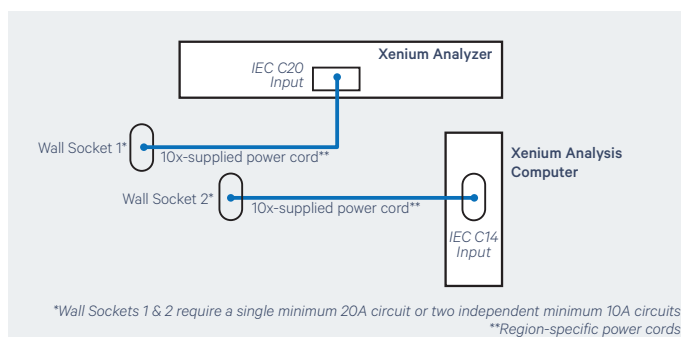


UPS should be plugged into an independent circuit. Refer to the user-acquired UPS installation documentation for UPS input circuit requirements.

Xenium Analyzer Configuration with UPS (recommended)



Xenium Analyzer Configuration without UPS



Refer to the [10x Genomics Support website](#) for the most current information regarding region-specific power cords.

Power Cords

10x Genomics will ship two region-specific power cords that are compatible with the regional wall sockets and the Xenium Analyzer IEC C20 input and the Xenium Analysis Computer IEC C14 input. If the instrument is connected to a UPS, region-specific compatible power cords in compliance with the local standards need to be acquired by the user.

Network Connectivity

Networking capabilities allow for egress of output files to shared network drives and enable faster troubleshooting via remote support of the Xenium Analyzer. Users will have the ability to enable and disable remote access to their instrument directly. The user needs to inform the IT department of their institution regarding the network/Internet access.

Refer to the [Xenium Analyzer Network Connectivity Guidelines Technical Note \(CG000645\)](#) for comprehensive information regarding remote performance monitoring and remote support along with additional technical details.

Contact support@10xgenomics.com for additional information.

1.4 Items for Installation & Training *(not supplied by 10x Genomics)*

Review the items listed below that should be available during on-site installation by a 10x Genomics representative. Refer to the manufacturer's website for regional part numbers.

For Installation				
Item		Description	Vendor	Part Number
<input type="checkbox"/>	Nuclease-free Water	Nuclease-free Water (not DEPC-treated)	Thermo Fisher Scientific	AM9932/ AM9937
		Nuclease-free Milli-Q water (Biopak® Polisher) <i>(select one based on availability)</i>	Millipore Sigma	CDUFBIOA1
<input type="checkbox"/>	PBS-T	Phosphate Buffered Saline with 0.05% Tween 20, pH 7.4 Phosphate Buffered Saline with 0.05% Tween 20, pH 7.4 <i>(select one based on availability)</i>	Millipore Sigma Millipore Sigma	P3563-10PAK PPB005-20PAK
<input type="checkbox"/>	PBS <i>Alternate for making PBS-T</i>	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
<input type="checkbox"/>	10% Tween 20	Tween 20 Surfact-Amps Detergent Solution (10% solution)	Thermo Fisher Scientific	28320
		10% Tween-20	Bio-Rad	1610781
<input type="checkbox"/>	100% DMSO	Dimethyl sulfoxide (molecular biology grade) Dimethyl sulfoxide (molecular biology grade) Dimethyl sulfoxide, Fisher BioReagents (>99.7%) Dimethyl sulfoxide (for molecular biology, 99.5+%) <i>(select one based on availability)</i>	Millipore Sigma Millipore Sigma Fisher Scientific Fuji Film	41639-500 ML D8418-1L BP231-1 043-29355 500 ml
<input type="checkbox"/>	KCl	Potassium Chloride (KCl, sterile), 500 ml Potassium Chloride (KCl, sterile), 1L KCl (2 M), RNase-free <i>(conc. in working solution will be 50 mM; select one based on availability)</i>	Teknova Teknova Invitrogen	P0330 P0335 AM9640G
Additional Materials				
<input type="checkbox"/>	Centrifuge	Allegra X-14 Benchtop Centrifuge 120 V <i>Discontd. or equivalent; fits deep-well 96 well plates (~2 ml vol.)</i>	Beckman Coulter Coulter	-
<input type="checkbox"/>	Serological Pipettes	10 ml, 25 ml, 50 ml, 100 ml		
<input type="checkbox"/>	Serological Pipette Controller	Compatible with 10, 25, 50 & 100 ml serological pipettes		
<input type="checkbox"/>	Graduated Cylinders	500 ml and 1 L		
<input type="checkbox"/>	Pipette Tips	Tips LTS 1ML Filter RT-L1000FLR <i>(or equivalent)</i>	Rainin	30389213
<input type="checkbox"/>	Pipettes	Pipet-Lite LTS Pipette L-1000XLS+ <i>(or equivalent)</i>	Rainin	17014382
<input type="checkbox"/>	Glass Bottles with Cap	Pyrex Reusable Media Storage Bottles (500 ml and 1 L) <i>(or equivalent)</i>		
<input type="checkbox"/>	Compressed Canned Air for cleaning			
<input type="checkbox"/>	Lens-cleaning Paper or Lint-free Laboratory Wipes			

Additional Materials

- ☐ Plate seals
- ☐ 70% Isopropanol
- ☐ Laboratory Balance
- ☐ Ultrapure/Milli-Q water, *from Milli-Q Integral Ultrapure Water System or equivalent*
- ☐ A fume hood is available during installation with appropriate procedures in place for handling volatile and hazardous chemicals in compliance with your institutional guidelines
- ☐ A liquid waste disposal system is available in compliance with your institutional guidelines
- ☐ If the Ethernet port on site cannot be reached using the 8 ft cable (10x-supplied), a CAT6 or higher Ethernet cable is available to connect to the port

This list may not include some standard laboratory equipment.

2.0 FFPE Samples

2.1 Workflow Documents

Documents will be available on the 10x Genomics Support website.

1 Tissue Preparation

Tissue Preparation Guide

Section FFPE tissue onto Xenium Slides

Demonstrated Protocol
CG000578

2 Fixation and Permeabilization

Fixation and Permeabilization

Deparaffinize and decrosslink FFPE tissue sections

Demonstrated Protocol
CG000580

3 Assay Workflow



Consult the relevant user guide depending on the workflow

Xenium In Situ Gene Expression*

For FF and FFPE samples on Xenium Slides

User Guide CG000582

OR

Xenium In Situ Gene Expression with Cell Segmentation Staining

For FF and FFPE samples on Xenium Slides

User Guide CG000749

4 Instrument

Xenium Analyzer

Load Xenium slide onto Xenium Analyzer

User Guide CG000584

5 Post-Instrument Activities (Optional)

H&E Staining (Optional)

H&E stain post-Xenium run

Demonstrated Protocol
CG000613

Consult the Post-Xenium In Situ Applications: Immunofluorescence, H&E, and Visium CytAssist Spatial Gene Expression Technical Note (CG000709) for additional applications.

2.2 FFPE Samples - Key Protocol Steps & Timing

» Tissue Sectioning & Section Placement (off-instrument; ~3 h)


Demonstrated Protocol CG000578



Sections placed on the Xenium slide can be stored at room temperature in a desiccator for up to 4 weeks.

» Tissue Section Deparaffinization & Decrosslinking (off-instrument; ~4.5 h)

Demonstrated Protocol CG000580

Steps		Timing
1.1	Buffer Preparation	30 min
1.2	Deparaffinization	3 h (includes 2 h baking step at 60°C)
1.3	Cassette Assembly	10 min
1.4	Decrosslinking	45 min
		 Proceed immediately to Probe Hybridization, Ligation & Amplification for Gene Expression

» Xenium In Situ Gene Expression (off-instrument; ~2 d)

User Guide CG000582

OR

» Xenium In Situ Gene Expression with Cell Segmentation Staining (off-instrument; ~3 d)

User Guide CG000749

Refer to the [Xenium In Situ Gene Expression](#) section for details.

» Xenium Analyzer (on-instrument; ~2-4 d)

Refer to the [Xenium Analyzer](#) section for details.

User Guide CG000584

2.3 FFPE Samples - Reagents & Consumables *(not supplied by 10x Genomics)*

FFPE Tissue Sectioning & Section Placement

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

For FFPE Tissue Sectioning & Section Placement				
	Item	Description	Vendor	Part Number
<input type="checkbox"/>	Microtome	Epredia HM 355S Automatic Microtome <i>or any standard histology grade microtome</i>	Fisher Scientific	23-900-672
<input type="checkbox"/>	Microtome blade	Epredia MX35 Premier Disposable Microtome Blades, Low Profile	Fisher Scientific	3052835
<input type="checkbox"/>	Cool-Cut, <i>Optional</i>	Thermo Scientific Cool-Cut	Fisher Scientific	77-112-0
<input type="checkbox"/>	Section transfer system (STS) <i>Optional</i>	Thermo Scientific Section Transfer System (STS), <i>Optional</i>	Fisher Scientific	771200
<input type="checkbox"/>	Probes	Fisherbrand Fine Precision Probe	Fisher Scientific	12-000-153
<input type="checkbox"/>	Forceps	Fisherbrand Curved Medium Point General Purpose Forceps	Fisher Scientific	16-100-110
<input type="checkbox"/>	Blank Slides <i>Optional, for sectioning practice</i>	Superfrost Plus Microscope Slides	Geyer	194242
<input type="checkbox"/>	Water bath	Tissue Floating Bath, Lighted <i>(or equivalent)</i>	Fisher Scientific	A84600061
		Epredia Digital Round Tissue Section Water bath <i>If using optional Section Transfer System</i>	Fisher Scientific	A84600061
<input type="checkbox"/>	Section dryer oven <i>Optional, but recommended</i>	Epredia High Capacity Section Dryer <i>or equivalent. Thermal cyclers may also be used for section drying</i>	Fisher Scientific	A84600051
<input type="checkbox"/>	Brushes	Camel Hair Brushes <i>(or equivalent)</i>	Ted Pella	11859
<input type="checkbox"/>	Fan <i>For drying slides</i>	Personal Rechargeable Fan <i>(or equivalent)</i>	Holmes	085-01-0117
<input type="checkbox"/>	Cutting Mat	WellTech Cutting Mat	WellTech Precision Lab	-
<input type="checkbox"/>	Wax Trimmer <i>Optional</i>	Electronic Microscopy Sciences Paraffin Block Trimmer Wax Trimmer, 115 VAC	Fisher Scientific	NC0310844
Additional Materials				
<input type="checkbox"/>	Razor blades			
<input type="checkbox"/>	Ice bucket (4–5 L)			
<input type="checkbox"/>	Ultrapure/Milli-Q Water for Water Bath, from Milli-Q Integral Ultrapure Water System or equivalent			

This list may not include some standard laboratory equipment.

FFPE Tissue Sections: Deparaffinization & Decrosslinking

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

For FFPE Tissue Sections: Deparaffinization & Decrosslinking			
Item	Description	Vendor	Part Number
<input type="checkbox"/> Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
<input type="checkbox"/> or	Xylene, Histological Grade	Millipore Sigma	534056
<input type="checkbox"/> Neo-clear	Neo-clear Xylene Alternative Substitute <i>Only tested for the Xenium Gene Expression workflow</i>	Millipore Sigma	1098435000
<input type="checkbox"/> Ethanol	Ethyl Alcohol, 200 Proof, anhydrous	Millipore Sigma	E7023
	Ethanol absolute ≥99.5%, TechniSolv, pure (Europe)	VWR	83813.360DP
<input type="checkbox"/> Nuclease-free Water	Nuclease-free Water (not DEPC-treated)	Thermo Fisher Scientific	AM9932/ AM9937
<input type="checkbox"/> PBS	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
<input type="checkbox"/> Urea	Urea Solution, 8M	Millipore Sigma	51457
<input type="checkbox"/> 10% Tween 20	Tween 20 Surfact-Amps Detergent Solution (10% solution; <i>not 100% Tween diluted to 10%</i>)	Thermo Fisher Scientific	28320
	10% Tween-20	Bio-Rad	1662404/ 1610781
<input type="checkbox"/> Forceps	Tweezers, 4" Wafer Handling	Excelta Corp	491P-SA-PI
<input type="checkbox"/> Staining jar/dishes	Coplin Jar	VWR	100500-232
	Staining Dishes	VWR	25608-906
<input type="checkbox"/> Section dryer oven	Epredia High Capacity Section Dryer <i>or equivalent. Thermal cyclers may also be used for section drying</i>	Fisher Scientific	A84600051
Additional Materials			
<input type="checkbox"/> Water Bath	<i>Alternatively, Eppendorf Thermomixer C (5382000023) with SmartBlock -2.0 mL (5362000035) or equivalent may be used</i>		
<input type="checkbox"/> Slide drying rack			
<input type="checkbox"/> Fume Hood			
<input type="checkbox"/> Vortex			
<input type="checkbox"/> Ultrapure/Milli-Q Water for Water Bath	<i>from Milli-Q Integral Ultrapure Water System or equivalent</i>		

This list may not include some standard laboratory equipment.

Refer to the [Xenium In Situ Gene Expression](#) section and the [Xenium Analyzer](#) section for reagents & consumables required. The information in these two sections applies to both FFPE and FF samples.

3.0 Fresh Frozen Samples

3.1 Workflow Documents

Documents will be available on the 10x Genomics Support website.

1 Tissue Preparation

Tissue Preparation Guide

Section FF tissue onto Xenium Slides

Demonstrated Protocol
CG000579

2 Fixation and Permeabilization

Fixation and Permeabilization

Deparaffinize and decrosslink FF tissue sections

Demonstrated Protocol
CG000581

3 Assay Workflow



Consult the relevant user guide depending on the workflow

Xenium In Situ Gene Expression*

For FF and FFPE samples on Xenium Slides

User Guide CG000582

OR

Xenium In Situ Gene Expression with Cell Segmentation Staining

For FF and FFPE samples on Xenium Slides

User Guide CG000749

4 Instrument

Xenium Analyzer

Load Xenium slide onto Xenium Analyzer

User Guide CG000584

5 Post-Instrument Activities (Optional)

H&E Staining (Optional)

H&E stain post-Xenium run

Demonstrated Protocol
CG000613

Consult the Post-Xenium In Situ Applications: Immunofluorescence, H&E, and Visium CytAssist Spatial Gene Expression Technical Note (CG000709) for additional applications.

3.2 Fresh Frozen Samples - Key Protocol Steps & Timing

» Tissue Sectioning & Section Placement (off-instrument; ~3 h)


Demonstrated Protocol CG000579



Sections placed on the Xenium slide can be stored at -80°C for up to 4 weeks.

» Tissue Section Fixation + Permeabilization (off-instrument; ~2.5 h)

Demonstrated Protocol CG000581

Steps		Timing
1.1	Buffer Preparation	30 min
1.2	Slide Preparation	5 min
1.3	Fixation	30 min
1.4	Permeabilization	65 min
1.5	Cassette Assembly	10 min
		Proceed immediately to Probe Hybridization, Ligation & Amplification for Gene Expression

» Xenium In Situ Gene Expression (off-instrument; ~2 d)

User Guide CG000582

OR

» Xenium In Situ Gene Expression with Cell Segmentation Staining (off-instrument; ~3 d)

User Guide CG000749

Refer to the [Xenium In Situ Gene Expression](#) section for details.

» Xenium Analyzer (on-instrument; ~2-4 d)

Refer to the [Xenium Analyzer](#) section for details.

User Guide CG000584

3.3 Fresh Frozen Samples - Reagents & Consumables *(not supplied by 10x Genomics)*

Fresh Frozen (FF) Tissue Sectioning & Section Placement

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

For FF Tissue Sectioning & Section Placement			
Item	Description	Vendor	Part Number
Tissue Freezing			
<input type="checkbox"/> Isopentane	Isopentane (2-Methylbutane)	Millipore Sigma	270342
<input type="checkbox"/> Forceps	Specimen Forceps, Straight, 203 mm (8")	VWR	82027-436
	Specimen Forceps, Straight, 152 mm (6")	VWR	82027-438
Frozen Tissue Embedding			
<input type="checkbox"/> Embedding Compound	TissueTek O.C.T. Compound	VWR	25608-930
<input type="checkbox"/> Embedding Molds	Epredia Peel-A-Way Disposable Embedding Molds	Fisher Scientific	12-20
Frozen Tissue Sectioning			
<input type="checkbox"/> Blank Slides <i>Optional, for sectioning practice</i>	Superfrost Plus Microscope Slides	Fisher Scientific	12-550-15
<input type="checkbox"/> Cryostat	CryoStar NX70 Cryostat	Fisher Scientific	957020
<input type="checkbox"/> Brushes	Flat cryostat brush, 10 mm <i>(or equivalent)</i>	Fisher Scientific	14-071-00
<input type="checkbox"/> Specimen Chuck	Thermo Scientific CryoStar NX70 Specimen Chuck	Fisher Scientific	14-071-413
<input type="checkbox"/> Microtome Blade	MX35 Ultra Microtome Blade, Low Profile	Fisher Scientific	3051835
<input type="checkbox"/> Slide Mailer	Simport Scientific LockMailer Tamper Evident Slide Mailer	Fisher Scientific	22-038-399
<input type="checkbox"/> Anti-Roll Plate <i>Optional</i>	Glass Anti-Roll Plate	Fisher Scientific	A78930200
Additional Materials			
<input type="checkbox"/> Dry Ice			
<input type="checkbox"/> Razor blades			
<input type="checkbox"/> Ice bucket			
<input type="checkbox"/> Aluminum Foil			

This list may not include some standard laboratory equipment.

Fresh Frozen (FF) Tissue Sections: Fixation & Permeabilization

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers

For FF Tissue Sections: Fixation & Permeabilization				
	Item	Description	Vendor	Part Number
<input type="checkbox"/>	PBS	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
<input type="checkbox"/>	Nuclease-free Water	Nuclease-free water (not-DEPC treated)	Thermo Fisher Scientific	AM9932/ AM9937
	Formaldehyde	Formaldehyde (37% by Weight/Molecular Biology)	Thermo Fisher Scientific	BP531-500
<input type="checkbox"/>	or	Formaldehyde solution	Millipore Sigma	252549/ F8775/ 47608
	Paraformaldehyde	Paraformaldehyde 16% Aqueous Solution, EM Grade	Electron Microscopy Sciences	15710
<input type="checkbox"/>	Ethanol	Ethyl Alcohol, 200 Proof, anhydrous	Millipore Sigma	E7023
		Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only)	VWR	83813.360DP
<input type="checkbox"/>	10% Tween-20	Tween 20 Surfact-Amps Detergent Solution (10% solution)	Thermo Fisher Scientific	28320
		10% Tween-20	Bio-Rad	1662404/ 1610781
<input type="checkbox"/>	Methanol	Methanol, for HPLC	Millipore Sigma	34860
<input type="checkbox"/>	SDS	Sodium dodecyl sulfate solution (for molecular biology, 10% in H2O)	Millipore Sigma	71736
<input type="checkbox"/>	Forceps	Tweezers, 4" Wafer Handling	Excelta Corp	491P-SA-PI
<input type="checkbox"/>	Slide Mailers	Sim port Scientific LockMailer Tamper Evident Slide Mailer	Fisher Scientific	22-038-399
Additional Materials				
<input type="checkbox"/>	Dry Ice	10x Genomics has tested only the listed thermal cyclers. Currently no alternate recommendations are available. Use one of the listed thermal cyclers based on preference and availability.		
<input type="checkbox"/>		<ul style="list-style-type: none">Bio-Rad: C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module (1851197)Analytik Jena: Biometra TAdvanced 96 SG (846-x-070-241 where x=2 for 230 V; 4 for 115 V; 5 for 100 V, 50-60 Hz)VWR: XT⁹⁶Gradient with 96-well gradient block & standard lid (76452-153)Marshall Scientific: MJ Research PTC-200 Thermal Cycler (05434-05)		
<input type="checkbox"/>	Slide drying rack			
<input type="checkbox"/>	Serological pipettes			
<input type="checkbox"/>	Fume Hood			
<input type="checkbox"/>	Vortex			
<input type="checkbox"/>	Ice bucket			
<input type="checkbox"/>	Ultrapure/Milli-Q Water for Water Bath	from Milli-Q Integral Ultrapure Water System or equivalent		

This list may not include some standard laboratory equipment.

Refer to the [Xenium In Situ Gene Expression](#) section and the [Xenium Analyzer](#) section for reagents & consumables required for these steps. The information applies to both FF and FFPE samples.

4.0 Xenium In Situ Gene Expression

with optional Cell Segmentation Staining

4.1 Key Protocol Steps & Timing (off-instrument; for both FFPE & FF samples)

» **Xenium In Situ Gene Expression (off-instrument; ~2 d)**

User Guide CG000582

OR

» **Xenium In Situ Gene Expression with Cell Segmentation Staining (off-instrument; ~3 d)**

User Guide CG000749

Steps		Timing	Stop & Store
Day 1			
Step 1: Probe Hybridization			
1.1	Buffer Preparation	20 min	
1.2	Probe Hybridization	16-24 h (overnight)	
Day 2			
Step 2: Post Hybridization Wash			
2.1	Post Hybridization Wash	35 min	
Step 3: Ligation			
3.1	Ligation	~2 h	
Step 4: Amplification			
4.1	Amplification	~2 h	
4.2	Post-Amplification wash	15 min	
<i>After Post-Amplification wash, if performing Cell Segmentation staining, Block and Stain step will require ~1 h followed by 16-24 h incubation. Next day Stain Enhancement step will be ~1 h followed by Autofluorescence Quenching.</i>			
Step 5: Autofluorescence Quenching			
5.1	Autofluorescence Quenching	30 min	 4°C overnight (in the dark)
5.2	Nuclei Staining	10 min	 4°C overnight or ≤1 week* (in the dark)



Refer to the relevant user guides for long-term slide storage guidance.

4.2 Xenium In Situ Gene Expression - Reagents & Consumables

(not supplied by 10x Genomics)

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

Xenium In Situ Gene Expression			
Item	Description	Vendor	Part Number
<input type="checkbox"/>	Nuclease-free water	Nuclease-free Water (not DEPC-Treated)	Thermo Fisher Scientific
		Nuclease-free Milli-Q water (Biopak® Polisher)	Millipore Sigma
		(select one based on availability)	AM9932/ AM9937 CDUFBIOA1
<input type="checkbox"/>	TE Buffer	TE Buffer, TRIS-EDTA, 1X Solution, pH 8.0	Fisher Scientific
<input type="checkbox"/>	PBS	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	BP24731
<input type="checkbox"/>	10% Tween 20	Tween 20 Surfact-Amps Detergent Solution (10% solution)	Thermo Fisher Scientific
		10% Tween-20	AM9624
<input type="checkbox"/>	Ethanol	Tween 20 Surfact-Amps Detergent Solution (10% solution)	28320
		10% Tween-20	Bio-Rad
<input type="checkbox"/>	Ethanol	Ethyl Alcohol, 200 Proof, anhydrous	1662404/ 1610781
		Ethanol absolute ≥99.5%, TechniSolv, pure (Europe)	Bio-Rad
	Glycerol	Glycerol, 99.5 % Molecular Biology, DNase, RNase, Protease free (optional, if storing slides long-term)	E7023-500ML
<input type="checkbox"/>	1.5 ml tubes	DNA LoBind Tubes, 1.5 ml	83813.360DP
		Low DNA Binding Tubes, 1.5 ml	Acros Organics
<input type="checkbox"/>	2.0 ml tubes	DNA LoBind Tubes, 2.0 ml	327255000
		Low DNA Binding Tubes, 2.0 ml	Eppendorf
<input type="checkbox"/>	15 ml tubes	15 ml PP Centrifuge Tubes	022431021
<input type="checkbox"/>	50 ml tubes	Self-Standing Polypropylene Centrifuge Tubes (50 ml), sterile	Sarstedt
<input type="checkbox"/>	Pipette tips	Tips LTS 200UL Filter RT-L200 FLR	72.706.700
		Tips LTS 1ML Filter RT-L1000 FLR (or equivalent)	Eppendorf
		Tips LTS 20UL Filter RT-L20 FLR	022431048
<input type="checkbox"/>	Pipettes	Pipet-Lite LTS Pipette L-20XLS+	Sarstedt
		Pipet-Lite LTS Pipette L-100XLS+ (or equivalent)	72.695.700
		Pipet-Lite LTS Pipette L-200XLS+	Corning
		Pipet-Lite LTS Pipette L-1000XLS+	730791
	Blank Slides	Superfrost Plus Slides (optional, if practicing Xenium Cassette Insert assembly)	Corning
	Forceps	Fisherbrand Curved Medium Point General Purpose Forceps (or equivalent)	430921
Additional Materials			
<input type="checkbox"/>	Water Bath	Alternatively, Eppendorf Thermomixer C (5382000023) with SmartBlock -2.0 mL (5362000035) or equivalent may be used	Rainin
<input type="checkbox"/>	Mini centrifuge		30389240
<input type="checkbox"/>	Vortex		30389213
<input type="checkbox"/>	Ice Bucket		30389226
<input type="checkbox"/>	Ultrapure/Milli-Q Water for Water Bath	from Milli-Q Integral Ultrapure Water System or equivalent	Rainin
<input type="checkbox"/>	PCR Sealing Film (optional, for storing slides long-term, for Xenium Cassette Insert Practice)		Rainin

This list may not include some standard laboratory equipment.

5.0 Xenium Analyzer

5.1 Key Protocol Steps & Timing *(on-instrument; for both FFPE & FF samples)*

» Xenium Analyzer (on-instrument; ~2-4 d)

User Guide CG000584

Steps	Timing	
	Hands-on Time	Total Time
Day 1		
Thaw Decoding Reagents	5 min	16-24 h (overnight)
Day 2		
Prepare Buffers	1 h	1 h
Initialize Instrument	-	5-10 min
Input Experimental Details	5-10 min	5-10 min
Load Instrument	~5 min	~5 min
Overview Scan	-	1 h
Select Region & Initiate Run	~10 min	~10 min
Day 4-6		
Run Time	-	2-4 days
Post-Run Cleanup	5 min	10 min

5.2 Xenium Analyzer - Reagents & Consumables (not supplied by 10x Genomics)

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

For Reagent Bottle Buffer Preparation				
Item	Description	Vendor	Part Number	
<input type="checkbox"/> Nuclease-free Water	Nuclease-free Water (not DEPC-treated)	Thermo Fisher Scientific	AM9932/ AM9937	
<input type="checkbox"/> PBS-T	Phosphate Buffered Saline with 0.05% Tween 20, pH 7.4	Millipore Sigma	P3563-10PAK	
	Phosphate Buffered Saline with 0.05% Tween 20, pH 7.4 <i>(select one based on availability)</i>	Millipore Sigma	PPB005-20PAK	
<input type="checkbox"/> PBS <i>Alternate for making PBS-T</i>	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624	
<input type="checkbox"/> 10% Tween 20	Tween 20 Surfact-Amps Detergent Solution (10% solution) <i>(use one ampule per use)</i>	Thermo Fisher Scientific	28320	
	10% Tween-20	Bio-Rad	1662404/ 1610781	
<input type="checkbox"/> 100% DMSO	Dimethyl sulfoxide (molecular biology grade)	Millipore Sigma	41639-500 ML	
	Dimethyl sulfoxide (molecular biology grade)	Millipore Sigma	D8418-250ML	
	Dimethyl sulfoxide (molecular biology grade)	Millipore Sigma	D8418-1L	
	Dimethyl sulfoxide, Fisher BioReagents (>99.7%)	Fisher Scientific	BP231-1	
	Dimethyl sulfoxide (for molecular biology, 99.5+%) <i>(select one based on availability)</i>	Fuji Film	043-29355 500 ml	
<input type="checkbox"/> KCl	Potassium Chloride (KCl, sterile), 500 ml	Teknova	P0330	
	Potassium Chloride (KCl, sterile), 1L	Teknova	P0335	
	KCl (2 M), RNase-free <i>(conc. in working solution will be 50 mM; select one based on availability)</i>	Invitrogen	AM9640G	
Additional Materials				
<input type="checkbox"/> Centrifuge	Allegra X-14 Series Benchtop Centrifuge 120 V or equivalent; fits deep-well 96 well plates (~2 ml vol.)	Beckman Coulter	-	
<input type="checkbox"/> Serological Pipettes	10 ml, 25 ml, 50 ml, 100 ml			
<input type="checkbox"/> Serological Pipette Controller	Compatible with 10, 25, 50 & 100 ml serological pipettes			
<input type="checkbox"/> Graduated Cylinders	100 ml and other volumes as needed			
<input type="checkbox"/> Pipette Tips	Tips LTS 1ML Filter RT-L1000FLR <i>(or equivalent)</i>	Rainin	30389213	
<input type="checkbox"/> Pipettes	Pipet-Lite LTS Pipette L-1000XLS+ <i>(or equivalent)</i>	Rainin	17014382	
<input type="checkbox"/> Glass Bottles with Cap	Pyrex Reusable Media Storage Bottles (500 ml and 1 l) <i>(or equivalent)</i>			
<input type="checkbox"/> Compressed Canned Air for cleaning				
<input type="checkbox"/> Lens-cleaning Paper or Lint-free Laboratory Wipes				
<input type="checkbox"/> Plate seal				
<input type="checkbox"/> 70% Isopropanol				
<input type="checkbox"/> Laboratory Balance				
<input type="checkbox"/> Ultrapure/Milli-Q water, from Milli-Q Integral Ultrapure Water System or equivalent				

This list may not include some standard laboratory equipment.

Appendix

Quencher Removal & H&E Staining - Reagents & Consumables (not supplied by 10x Genomics)

Optional; Only if following 10x Genomics H&E protocol

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

For Quencher Removal				
Post-run quencher removal is required only if staining slides after the instrument run				
Item	Description	Vendor	Part Number	
<input type="checkbox"/> Sodium Hydrosulfite	Sodium hydrosulfite, technical grade <i>(or equivalent)</i>	Sigma Aldrich	157953-5G	
<input type="checkbox"/> Forceps	Tweezers, 4' Water Handling	Excelta Corp	491P-SA-PI	
<input type="checkbox"/> PBS <i>(optional)</i>	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624	
<input type="checkbox"/> Slide Mailers	Sim port Scientific LockMailer Tamper Evident Slide Mailer	Fisher Scientific	22-038-399	

For H&E Staining				
Item	Description	Vendor	Part Number	
<input type="checkbox"/> Hematoxylin	Hematoxylin Solution, Mayer's	Sigma Aldrich	MHS16	
<input type="checkbox"/> Eosin	Eosin Y Solution, Alcoholic	Leica	3801615	
<input type="checkbox"/> Bluing Reagent	Bluing Solution	Dako	CS702	
<input type="checkbox"/> Mounting Media	Surgipath SUB-X Mounting Media <i>Discontd.</i> Epredia Cytoseal Mountant <i>Cytoseal or equivalent mounting media can be used</i>	Leica Fisher Scientific	3801741 22-050-262	
<input type="checkbox"/> Ethanol	Ethyl Alcohol, 200 Proof, anhydrous	Millipore Sigma	E7023	
	Ethanol absolute ≥99.5%, TechniSolv, pure (Europe)	VWR	83813.360DP	
<input type="checkbox"/> Xylene	Xylene, Reagent Grade	Millipore Sigma	214736	
	Xylene, Histological Grade	Millipore Sigma	534056	
<input type="checkbox"/> Forceps	Tweezers, 4' Water Handling	Excelta Corp	491P-SA-PI	
<input type="checkbox"/> Filter Paper	Fisherbrand Qualitative Grade Plain Filter Paper Circles <i>or equivalent</i>	Fisher Scientific	09-795-H	
<input type="checkbox"/> Coverslips	Fisherbrand Cover Glasses: Rectangles <i>Discontinued</i>	Fisher Scientific	12-544-EP	
	Cover Glasses, Rectangles	VWR	16004-322	

Additional Materials

- ☐ Vortex
- ☐ Staining jar/dishes
- ☐ Wide-bore pipette tips
- ☐ Ultrapure/Milli-Q water, from Milli-Q Integral Ultrapure Water System or equivalent

This list may not include some standard laboratory equipment.

Document Revision Summary

Document Number	CG000601
Title	Xenium In Situ Gene Expression - Protocol Planner
Revision	Rev D to Rev E
Revision Date	March 2024

Specific Changes

- Updated introduction & workflow figure to include cell segmentation (page 1)
- Updated Vibration Isolation Table dimensions (page 4)
- Included Xenium In Situ Gene Expression with Cell Segmentation Staining User Guide for reference (multiple pages)
- Updated to include Technical Note CG000709 for reference in workflow documents (pages 11, 15)
- Updated protocol steps & timing and reagents required for the cell segmentation staining (pages 19, 20)

General Changes

- Updated to refer to the off-instrument workflow as "Xenium In Situ Gene Expression" or "Xenium In Situ Gene Expression with Cell Segmentation Staining"
- Updated for general minor consistency of format, language, and terms throughout

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Contact:

support@10xgenomics.com

10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588 USA

