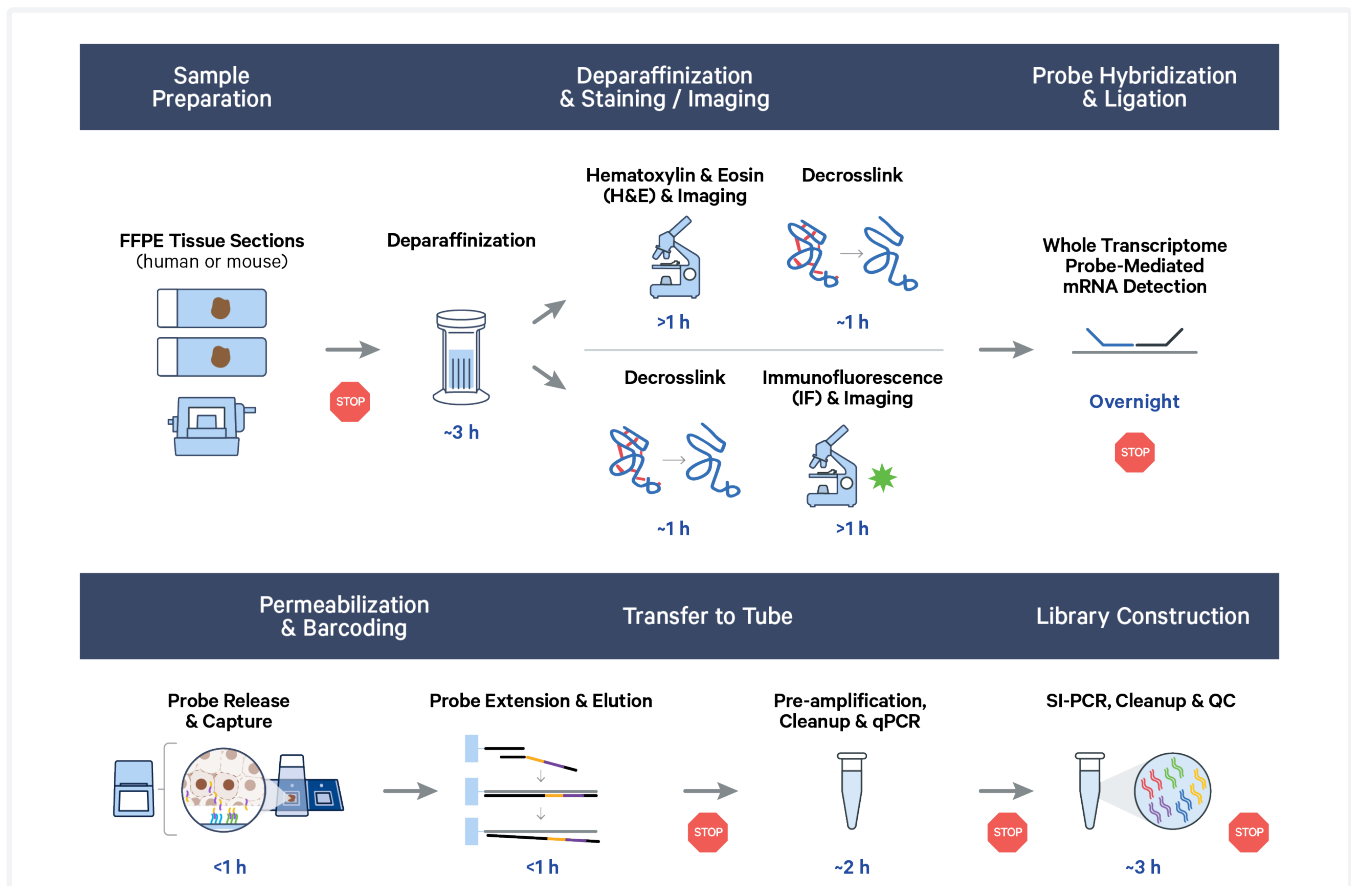


# Visium CytAssist Spatial Gene Expression for FFPE – Protocol Planner

## Introduction

Visium CytAssist Spatial Gene Expression for FFPE is designed to analyze mRNA expression in tissue sections derived from formalin fixed & paraffin embedded (FFPE) tissue samples. The Visium CytAssist instrument requires glass slides with intact tissue sections as input. This document provides a workflow overview and additional equipment, kits, and reagents for the Visium CytAssist Spatial Gene Expression for FFPE protocols. 10x Genomics Visium CytAssist Spatial Gene Expression for FFPE Kits are not listed in this document.

## Workflow Overview - Visium CytAssist Spatial Gene Expression for FFPE



## Kits, Reagents & Equipment

The items in the tables below have been validated by 10x Genomics and are highly recommended for the Visium Spatial Reagent Kits for FFPE. **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.

### Pipette and Pipette Tip Recommendations

Supplier	Description	Part Number (US)
Rainin (pipettes)	<input type="checkbox"/> Pipet-Lite Multi Pipette L8-200XLS+	17013805
	<input type="checkbox"/> Pipet-Lite LTS Pipette L-2XLS+	17014393
	<input type="checkbox"/> Pipet-Lite LTS Pipette L-10XLS+	17014388
	<input type="checkbox"/> Pipet-Lite LTS Pipette L-20XLS+	17014392
	<input type="checkbox"/> Pipet-Lite LTS Pipette L-100XLS+	17014384
	<input type="checkbox"/> Pipet-Lite LTS Pipette L-200XLS+	17014391
	<input type="checkbox"/> Pipet-Lite LTS Pipette L-1000XLS+	17014382
Rainin (tips)	<input type="checkbox"/> Tips LTS 200UL Filter RT-L200FLR	30389240
	<input type="checkbox"/> Tips LTS 1ML Filter RT-L1000FLR	30389213
	<input type="checkbox"/> Tips LTS 20UL Filter RT-L10FLR	30389226
Wide Bore Pipette tips	<input type="checkbox"/> Tips RT LTS 200UL FLW	30389241
	<input type="checkbox"/> Tips RT LTS 1000UL FLW	30389218

## Sample Preparation

For FFPE Tissue Sectioning & Section Placement			
Item	Alternatives/Options	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, <i>Optional</i>	Fisher Scientific	77-112-0
<input type="checkbox"/> Section transfer system (STS) <i>Optional</i>	Thermo Scientific Section Transfer System (STS), <i>Optional - If using Section Transfer System</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"/> Water bath	Premiere Tissue Floating Bath, Lighted <i>Or any equivalent water bath</i>	Fisher Scientific	A84600061
<input type="checkbox"/> Section dryer oven	Epredia High Capacity Section Dryer	Fisher Scientific	A84600051
<input type="checkbox"/> Slide drying rack <i>If using section dryer oven</i>	StatLab TISSUE-TEK VERT 24 SLIDE RACK	Fisher Scientific	LWS2124
<input type="checkbox"/> Thermal cycler <i>Alternative to section dryer oven</i>	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	Bio-Rad	1851197
<input type="checkbox"/> Brushes	Camel Hair Brushes, <i>Or any equivalent paintbrush</i>	Ted Pella	11859
<input type="checkbox"/> Blank Slides	Epredia Shandon ColorFrost Plus Slides	Fisher Scientific	6776214
	Fisherbrand Premium Superfrost Microscope Slides	Fisher Scientific	12-544-7
	Poly-Prep Slides	Millipore Sigma	P0425
	Superfrost Slides	VWR	47311-703
Additional Materials			
<input type="checkbox"/> Razor blades		-	-
<input type="checkbox"/> Ice bucket		-	-
<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.*

## RNA Quality Assessment Using DV200

For RNA Quality Assessment			
<input type="checkbox"/> Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
	Xylene, Histological Grade	Millipore Sigma	534056
<input type="checkbox"/> Ethanol	Ethyl Alcohol, Pure (200 Proof, anhydrous)	Millipore Sigma	E7023-500ML
	Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only)	VWR	83813.360DP
<input type="checkbox"/> RNA extraction kit	RNeasy FFPE Kit (50)	Qiagen	73504
	RNAsort Kit for Isolation of RNA	Biotium	CD501
	PureLink FFPE RNA Isolation Kit	Thermo Fisher Scientific	156002
<input type="checkbox"/> Deparaffinization solution	Deparaffinization Solution	Qiagen	19093
<input type="checkbox"/> RNase decontamination solution	RNaseZap RNase Decontamination Solution	Thermo Fisher Scientific	AM9780
<input type="checkbox"/> DNase Treatment	DNase Booster Buffer	Qiagen	1064143
	DNase I Solution	Thermo Fisher Scientific	89836
<input type="checkbox"/> Nuclease-free water	Nuclease-free Water (not DEPC-Treated)	Thermo Fisher Scientific	AM9937
<input type="checkbox"/> Staining dishes <i>If working with hard coverslipped slides</i>	Staining Dish, White	VWR	25608-906
<input type="checkbox"/> Metal Block <i>If working with hard coverslipped slides</i>	Light Labs 96-WELL ALUMINUM BLOCK	Fisher Scientific	NC9820161
<input type="checkbox"/> 1.5-ml centrifuge tubes	DNA LoBind Tubes, 1.5 ml	Eppendorf	022431021
<input type="checkbox"/> 0.2 ml PCR 8-tube strips  <i>Choose either Eppendorf, USA Scientific, or Thermo Fisher Scientific PCR 8-tube strips</i>	PCR Tubes 0.2 ml 8-tube strips	Eppendorf	951010022
	TempAssure PCR 8-tube strip	USA Scientific	1402-4700
	MicroAmp 8-TubeStrip, 0.2 ml	Thermo Fisher Scientific	N8020580
	MicroAmp 8-cap Strip, clear	Thermo Fisher Scientific	N8010535
<input type="checkbox"/> Nanodrop/Qubit fluorometer	Nanodrop 2000c Spectrophotometers Or any equivalent Nanodrop Alternative to Qubit Fluorometer	Thermo Fisher Scientific	ND-2000C
	Qubit RNA BR Assay Kit	Thermo Fisher Scientific	Q10210
	Qubit Assay Tubes	Thermo Fisher Scientific	Q32856
	Qubit 4 Fluorometer	Thermo Fisher Scientific	Q33238
<input type="checkbox"/> Bioanalyzer/Tapestation  <i>Choose Bioanalyzer or TapeStation based on availability &amp; preference.</i>	RNA 6000 Pico Kit	Agilent	5067-1513
	2100 Bioanalyzer Laptop Bundle	Agilent	G2953CA
	4200 TapeStation	Agilent	G2991AA
	High Sensitivity RNA ScreenTape	Agilent	5067-5579
	High Sensitivity RNA ScreenTape Ladder	Agilent	5067-5581
	High Sensitivity RNA ScreenTape Sample Buffer	Agilent	5067-5580

*This list may not include some standard laboratory equipment*

## Deparaffinization, H&E Staining, & Decrosslinking

For items with multiple options listed, choose option based on availability and preference.

Item	Alternatives/Options	Vendor	Part Number
<input type="checkbox"/> Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
	Xylene, Histological Grade	Millipore Sigma	534056
<input type="checkbox"/> Ethanol	Ethyl Alcohol, 200 Proof	Millipore Sigma	E7023
	Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only)	VWR	83813.360DP
<input type="checkbox"/> Eosin	Eosin Y-solution, Alcoholic	Millipore Sigma	HT110116
	Eosin Y Solution (Modified Alcoholic)	Abcam	ab246824
	Eosin Y with Phloxine 1% alcoholic solution	VWR	10143
<input type="checkbox"/> Hematoxylin	Hematoxylin Solution, Mayer's	Millipore Sigma	MHS16
	Hematoxylin Solution According to Mayer	Millipore Sigma	51275
	Hematoxylin, Mayer's	Agilent	S330930-2
<input type="checkbox"/> Bluing reagent	Bluing Reagent, Dako	Agilent	CS70230-2
	Thermo Scientific Shandon Bluing Reagent	Fisher Scientific	6769001
	Scott's Bluing Agent	Ricca Chemical Company	6697
<input type="checkbox"/> Glycerol	Glycerol Solution	Millipore Sigma	49781
	Glycerol	Acros Organics	327255000
<input type="checkbox"/> 0.1 N HCl	Hydrochloric Acid Solution, 0.1 N <i>Or any equivalent HCl</i>	Fisher Chemical	SA54-1
<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 any equivalent product. Thermal cyclers may also be used for section drying.</i>	Fisher Scientific	A84600051
<input type="checkbox"/> Slide holders	Slide Holders, 24-place	VWR	25608-868
<input type="checkbox"/> Coverslips (50 x 24 mm)	Fisherbrand Cover Glasses: Rectangles	Fisher Scientific	12-544-EP
	Cover Glasses, Rectangular	VWR	16004-322
Additional Materials			
<input type="checkbox"/> Beakers		-	-
<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.*

## Deparaffinization, Decrosslinking, Immunofluorescence Staining & Imaging

Item	Alternatives/Options	Vendor	Part Number
<input type="checkbox"/> Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
	Xylene, Histological Grade	Millipore Sigma	534056
<input type="checkbox"/> Glycerol	Glycerol Solution	Millipore Sigma	49781
	Glycerol	Acros Organics	327255000
<input type="checkbox"/> Ethanol	Ethyl Alcohol, 200 Proof	Millipore Sigma	E7023
	Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only)	VWR	83813.360DP
<input type="checkbox"/> PBS	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
<input type="checkbox"/> Nuclease-free water	Nuclease-free Water (not DEPC-Treated)	Thermo Fisher Scientific	AM9937
<input type="checkbox"/> DAPI	DAPI Solution (1 mg/ml)	Thermo Fisher Scientific	62248
<input type="checkbox"/> RNase inhibitor	Protector RNase Inhibitor	Millipore Sigma	3335399001
<input type="checkbox"/> Mounting medium	SlowFade Diamond Antifade Mountant	Thermo Fisher Scientific	S36967
	SlowFade Gold Antifade Mountant	Thermo Fisher Scientific	S36966
<input type="checkbox"/> Section dryer oven	Epredia High Capacity Section Dryer	Fisher Scientific	A84600051
<input type="checkbox"/> Slide holders	Slide Holders, 24-place	VWR	25608-868
<input type="checkbox"/> Coverslips (50 x 24 mm)	Fisherbrand Cover Glasses: Rectangles	Fisher Scientific	12-544-EP
	Cover Glasses, Rectangular	VWR	16004-322
<input type="checkbox"/> Staining jar/dishes	Coplin Jar	VWR	100500-232
	Staining Dishes	VWR	25608-906
<input type="checkbox"/> Autofluorescence Quencher	TrueBlack Plus Lipofuscin Autofluorescence Quencher <i>Optional</i>	Biotium	23014
Additional Materials			
<input type="checkbox"/> Beakers		-	-
<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.*

## Imaging for FFPE

**An imaging system** with a scanning stage that can accurately capture and stitch together large tiled images is recommended to run Visium Spatial for FFPE. The microscope objective should be dry and free of immersion oil. Low magnification, high Numeric Aperture (NA) microscopes, e.g., 10x/0.4NA or 20x/0.8NA, are recommended to balance high resolution and high acquisition speed. Slides are loosely mounted with glycerol and a coverslip and therefore should not be inverted for imaging. If using an inverted microscope, perform imaging through the slide rather than the coverslip and use an objective (with a correction collar) designed to image through microscope slides.

Supplier	Description	Configuration
Thermo Fisher Scientific	EVOS M7000	Inverted
Leica	Aperio Versa 8 Leica DMI8	Upright 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 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 *May vary depending on imaging system*

### Fluorescence Configuration (if performing IF staining)

Light source (or equivalent) with a wavelength range of 380-680 nm

Monochrome camera (14 bit, 2,424 x 2,424 pixel resolution)

DAPI filter cube (Excitation 392/23, Emission 447/60)

Additional filters - select based on fluorophore/antibodies used

2.18 µm/pixel minimum capture resolution

Exposure times 100 milli sec - 2 sec *May vary depending on imaging system*

### Additional Reagents and Consumables for the Visium CytAssist Spatial Gene Expression for FFPE Assay

The items in the table below have been validated by 10x Genomics and are highly recommended for the Visium Spatial Reagent Kits for FFPE. **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.

Item	Description	Vendor	Part Number
<b>Plastics</b>			
<input type="checkbox"/> 1.5 ml tubes	DNA LoBind Tubes, 1.5 ml	Eppendorf	022431021
	Low DNA Binding Tubes, 1.5 ml	Sarstedt	72.706.700
<input type="checkbox"/> 2.0 ml tubes	DNA LoBind Tubes, 2.0 ml when processing more than two slides	Eppendorf	022431048
	Low DNA Binding Tubes, 2.0 ml	Sarstedt	72.695.700
<input type="checkbox"/> 15 ml tubes	15 ml PP Centrifuge Tubes	Corning	730791
<input type="checkbox"/> 50 ml tubes	Self-Standing Polypropylene Centrifuge Tubes (50 ml), sterile	Corning	430921
<input type="checkbox"/> 0.2 ml PCR 8-tube strips	PCR Tubes 0.2 ml 8-tube strips	Eppendorf	951010022
	TempAssure PCR 8-tube strip	USA Scientific	1402-4700
	MicroAmp 8-Tube Strip, 0.2 ml	Thermo Fisher Scientific	N8010580
	MicroAmp 8-Cap Strip, clear	Thermo Fisher Scientific	N8010535
<input type="checkbox"/> Slide mailer/tube	Simport Scientific LockMailer Tamper Evidence Slide Mailer	Thermo Fisher Scientific	22-038-399
	Self-Standing Polypropylene Centrifuge Tubes (50 ml), sterile <i>Alternative to slide mailer</i>	Corning	430921
<input type="checkbox"/> PCR plates and sealing film	Hard-shell PCR Plates 96-well, thin wall (pkg of 50) Or any compatible PCR Plate	Bio-Rad	HSP9665
	Microseal 'B' PCR Plate Sealing Film, adhesive	Bio-Rad	MSB1001
<input type="checkbox"/> Reagent reservoirs	Divided Polystyrene Reservoirs	VWR	41428-958
<b>Kits &amp; Reagents</b>			
<input type="checkbox"/> Nuclease-free water	Nuclease-free Water (not DEPC-Treated)	Thermo Fisher Scientific	AM9937
<input type="checkbox"/> Tris 1 M (Tris-HCl)	Tris 1 M, pH 8.0, RNase-free	Thermo Fisher Scientific	AM9850G
	Tris 1 M, pH 8.0	Teknova	T5080
	UltraPure 1M Tris-HCl, pH 8.0	Invitrogen	15568025
<input type="checkbox"/> Blank Slides	Shandon ColorFrost Plus Slides 25 x 75 x 1 mm	Thermo Fisher Scientific	12-544-4
	Fisherbrand Premier Plain Glass Microscope Slides	Thermo Fisher Scientific	12-544-4
<input type="checkbox"/> 10X PBS	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
<input type="checkbox"/> Tween 20	Tween 20 Surfact-Amps Detergent Solution (10% solution)	Thermo Fisher Scientific	28320



### Additional Reagents and Consumables for the Visium CytAssist Spatial Gene Expression for FFPE Assay continued

<input type="checkbox"/>	qPCR Mix	KAPA SYBR FAST qPCR Master Mix (2X)	Roche (United States, some Canadian Provinces) Millipore Sigma (Europe, Asia, & Canada)	KK4600
<input type="checkbox"/>	SPRIselect reagent	SPRIselect Reagent Kit	Beckman Coulter	B23318
<input type="checkbox"/>	Ethanol	Ethanol, Pure (200 Proof, anhydrous)	Millipore Sigma	E7023-500ML
<input type="checkbox"/>	8M KOH	Potassium Hydroxide Solution, 8M	Millipore Sigma	P4494-50ML
<input type="checkbox"/>	20X SSC Buffer	SSC Buffer 20X Concentrate	Millipore Sigma	S66391L
<input type="checkbox"/>	Buffer EB	Qiagen Buffer EB	Qiagen	19086
<input type="checkbox"/>	Eosin	Eosin Y-solution, Alcoholic	Millipore Sigma	HT110116
		Eosin Y Solution (Modified Alcoholic)	Abcam	ab246824
		Eosin Y with Phloxine 1% alcoholic solution	VWR	10143
<input type="checkbox"/>	Ultrapure Water	Ultrapure/Milli-Q water		
<input type="checkbox"/>	Mini Centrifuge	VWR Mini Centrifuge Or any equivalent mini centrifuge	VWR	76269-064
<b>Quantification &amp; Quality Control</b>				
<input type="checkbox"/>	Bioanalyzer & associated reagents	2100 Bioanalyzer Laptop Bundle (discontinued)	Agilent	G2943CA
		Replacement 2100 Bioanalyzer Instrument/ 2100 Expert Laptop Bundle	Agilent	G2939BA/ G2953CA
		High Sensitivity DNA Kit	Agilent	5067-4626
<input type="checkbox"/>	TapeStation & associated reagents	4200 TapeStation	Agilent	G2991AA
		High Sensitivity D1000: ScreenTape/ Reagents	Agilent	5067-5592/ 5067-5593
		High Sensitivity D5000: ScreenTape/ Reagents	Agilent	5067-5584/ 5067-5585
<input type="checkbox"/>	LabChip & associated reagents	LabChip GX Touch HT Nucleic Acid Analyzer	PerkinElmer	CLS137031
		DNA High Sensitivity Reagent Kit	PerkinElmer	CLS760672
<input type="checkbox"/>	Qubit & associated reagents (for determining dilution factor for TapeStation, Bioanalyzer, or LabChip)	Qubit Fluorometer	ThermoFisher Scientific	Q33238
		Qubit Assay Tubes Reagents	ThermoFisher Scientific	Q32856
		Qubit dsDNA HS and BR Assay Kits	ThermoFisher Scientific	Q32854
<input type="checkbox"/>	Library Quantification Kit	KAPA Library Quantification Kit for Illumina Platforms	Roche (United States, some Canadian Provinces) Millipore Sigma (Europe, Asia, & Canada)	KK4824

Choose Bioanalyzer, TapeStation or LabChip based on availability & preference.

### 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

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

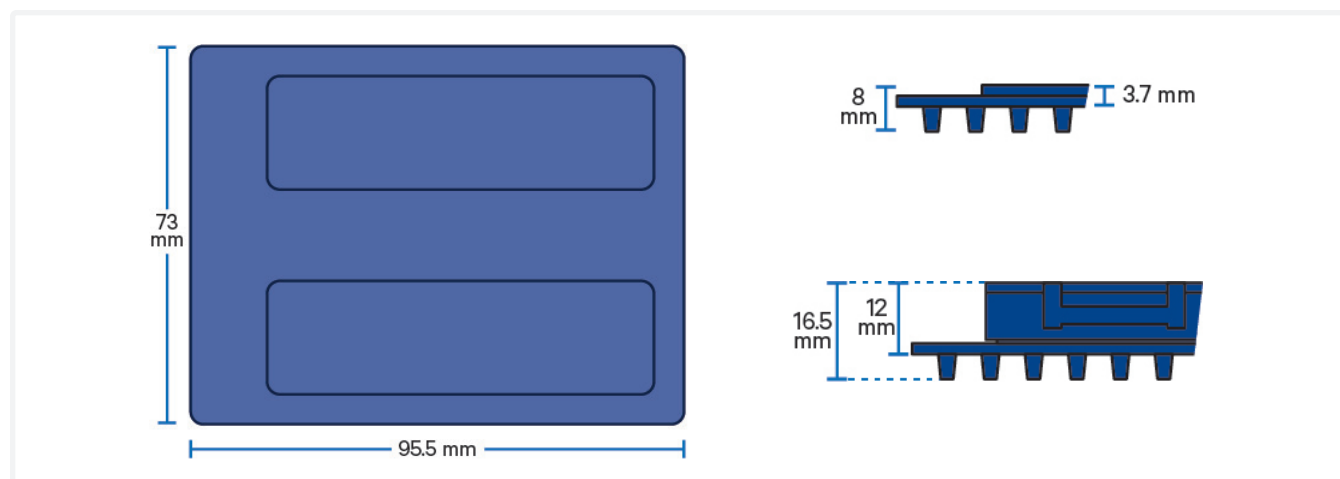
- Eppendorf MasterCycler X50s: 2°C/sec heating and cooling

Thermal cycler requirements if reactions are performed on a slide:

- Thermal cycler must be able to accommodate 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

Thermal cycler requirements if reactions are performed in a tube (Elution, Step 3.0 onward):

- Uniform heating of 100 µl volumes
- Temperature-controlled lid
- 96 deep-well block or 0.2 ml block configuration



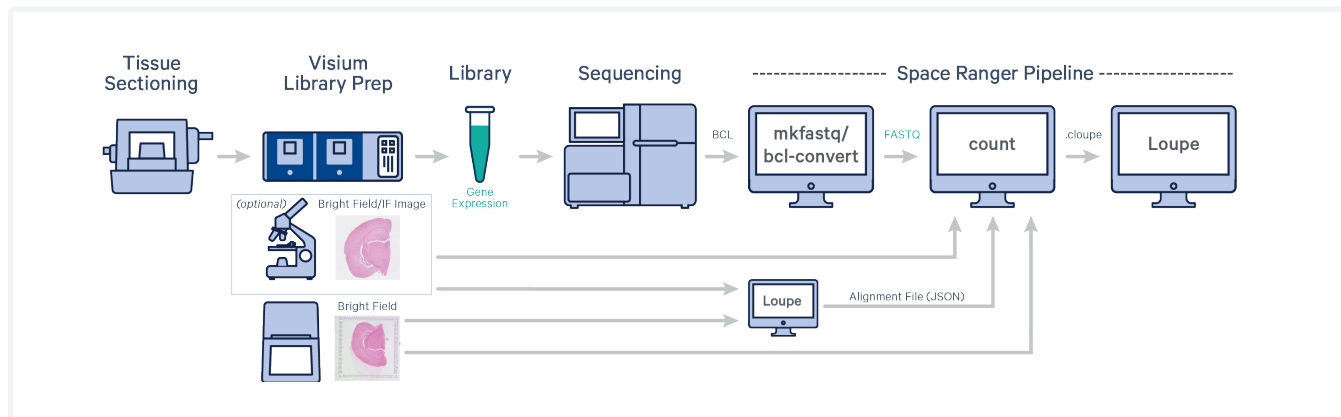
### Recommended Real Time qPCR Systems

Supplier	Description	Part Number
Applied Biosystems	QuantStudio 12K Flex System	4471087
Bio-Rad	CFX96 Real-time System (discontinued)	1855096

## Analysis of Visium CytAssist Spatial Gene Expression Data

For detailed information on analysis of Visium CytAssist Spatial Gene Expression data, refer to 10x Genomics [Software Support](#) site.

### Data Analysis Overview – Visium CytAssist Spatial Gene Expression for FFPE



Key information that should be collected and provided to the data analyst/bioinformatician to ensure that parameters are set appropriately for Space Ranger to analyze the data:

- Sample name, library type, and either Illumina adaptor index set or index sequences
- If a microscope image of the tissue section on plain glass is provided, then the corresponding imaging type (Brightfield vs Fluorescence (color composite) vs Fluorescence (multipage TIFF) for the supplied image
- Visium slide serial number and Capture Area on the slide corresponding to each sample
- The CytAssist instrument captured Brightfield image with fiducial frame

## Document Revision Summary

<b>Document Number</b>	CG000556
<b>Title</b>	Visium CytAssist Spatial Gene Expression - Protocol Planner
<b>Revision</b>	Rev B
<b>Revision Date</b>	July 2022
<b>General Changes</b>	Updated for general minor consistency of language and terms throughout
<b>Specific Changes</b>	<ul style="list-style-type: none"> <li>• Updated workflow diagram on (cover page)</li> <li>• Added slide drying rack and thermal cycler recommendations to sample preparation table (page 3)</li> <li>• Added xylene, staining dishes, metal block, and PCR 8-tube strip recommendations to RNA quality assessment table (page 4)</li> <li>• Added eosin to additional reagents and consumables table (page 9)</li> <li>• Updated recommended thermal cyclers (page 10)</li> </ul>

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