Mar 20, 2024

10 X Visium Spatial Gene Expression - Fixed Frozen Tissue Processing with CytAssist

DOI
https://dx.doi.org/10.17504/protocols.io.q26g71z49gwz/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.q26g71z49gwz/v1

External link: https://cdn.10xgenomics.com/image/upload/v1680564483/support-documents/CG000662_Demonstrated_Protocol_VisiumCytAssist_FixedFrozen_H_E_RevA.pdf

Protocol Citation: Alberto Pappalardo, Kavya Batra, Rolando Perez-Lorenzo, Angela Christiano 2024. 10 X Visium Spatial Gene Expression - Fixed Frozen Tissue Processing with CytAssist. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g71z49gwz/v1

Protocol status: Working
We use this protocol and it's working

Created: March 19, 2024

Last Modified: March 20, 2024

Protocol Integer ID: 96879

Keywords: performing 10x visium spatial gene expression, 10x visium spatial gene expression, visium spatial gene expression, fixed frozen tissue processing with cytassist, visium cytassist device, 10x genomic, 10x genomics website, fixed frozen tissue processing, frozen tissue section, gene expression, tissue section

Funders Acknowledgements:
NCI
Grant ID: 5UG3CA275686

Abstract

Here we summarize the reccomendation released by 10X Genomics for processing fixed-frozen tissue sections while performing 10X Visium Spatial Gene Expression assay using the Visium CytAssist device. Please follow the link for the detailed official protocol on 10X Genomics website.
https://cdn.10xgenomics.com/image/upload/v1680564483/support-documents/CG000662_Demonstrated_Protocol_VisiumCytAssist_FixedFrozen_H_E_RevA.pdf

Materials

Reagents

Visium FFPE Reagent Kit PN-1000436

Store at -20°C

	A	B
	Item	**Part number**
	Amp Mix B	2000567
	Extension Enzyme	2000389
	Extension Buffer	2000409
	RNase Enzyme	3000593
	RNase Buffer B	2000551
	Tissue Removal Enzyme	3000387
	Tissue Removal Buffer B	2000543
	Tissue Removal Buffer Enhancer	2000557
	Decrosslinking Buffer	2000566
	TS Primer Mix B	2000537
	Block and Stain Buffer	2000554

Visium FFPE Reagent Kit PN-1000436

Other Specific Reagents

	A	B	C	D
	Item	**Alternatives/Options**	**Vendor**	**Part Number**
	Ethanol	Ethyl Alcohol, 200 Proof	Millipore Sigma	E7023
		Ethanol absolute ≥99.5%	VWR	83813.360DP
	Eosin	Eosin Y-solution, Alcoholic	Millipore Sigma	HT110116
	Hematoxylin	Hematoxylin Solution, Mayer's	Millipore Sigma	MHS16
	Bluing Reagent	Bluing Reagent, Dako	Agilent	CS70230-2

	A	B	C	D
	1X PBS	Phosphate-Buffered Saline, 1X without calcium and magnesium, PH 7.4	Corning	21-040-CV
	Glycerol	Glycerol Solution	Millipore Sigma	49781
	0.1 N HCl	Hydrochloric Acid Solution, 0.1 N	Fisher Chemical	SA54-1

Other Materials

Visium CytAssist Slide and Cassettes, 6.5 mm PN-1000519 for 2 runs

Store at ambient temperature

	A	B	C
	Item	Quantity	Part number
	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

Visium CytAssist Slide and Cassettes, 6.5 mm
PN-1000519

Equipment

Thermal Cycler

Low Profile Plate Insert PN-3000823

10x Magnetic Separator PN-120250

Rehydration

1 **Rehydration**

1.1 Place a Low Profile Thermocycler Adapter on a thermal cycler and preheat thermal cycler to 37°C.

1.2 Retrieve the slides holding the tissue from the -80 ºC freezer. The tissue sections should be 10 µm thick and placed within the allowable area for the CytAssist device. For Fisherbrand Superforst slides the allowable area is a rectangle with the lenght sides distant 5 mm from the slide edge, and with the width sides distant 15 mm from the frosted area and the plus signs.

1.3 Place slide on the Low Profile Thermocycler Adapter with the tissue side facing up. Dry for 10 min at 37°C keeping the thermal cycler lid open.

1.4 Submerge the slide in a tube containg PBS for 5 min.

1.5 Submerge the slide slowly in a new tube containing Milli-Q Water for 3 min.

1.6 Submerge the slide slowly in a new tube containing 100% Ethanol for 3 min.

1.7 Submerge the slide slowly in a new tube containing 70% Ethanol for 3 min.

1.8 Submerge the slide slowly in a new tube containing Milli-Q Water for 20 sec.

1.9 Proceed immediately to H&E Staining & Coverslipping.

H&E Staining

2 **H & E staining**

2.1 Prepare 6 water beaker with Milli-Q Water to dip the slide.

2.2 Place slide on a flat impermeable staining surface.

2.3 Cover the slide with 1 ml of Hematoxylin making sure to uniformly cover the tissue section.

2.4 Incubate for 3 min at room temperature.

2.5 Remove the Hematoxylin and submerge the slide 5x in a water beaker (I).

2.6 Submerge the slide 15x in a new water beaker (II).

2.7 Submerge slide 15x in a new water beaker (III).

2.8 Place slide on back on the staining surface and add 1 ml of Bluing Buffer making sure to uniformly cover the tissue section. Incubate 1 min at room temperature.

2.9 Remive the Bluing Buffer and submerge the slide 10x in a new water beaker (IV).

2.10 Place slide on back on the staining surface and add 1 ml of Eosin making sure to uniformly cover the tissue section. Incubate 1 min at room temperature. DO NOT use diluted Eosin.

2.11 Remove the Eosin and submerge slide for 30 sec in a new water beaker (V).

2.12 Submerge the slide 10x in a new water beaker (VI)

Coverslipping

3 **Coverslipping**

3.1 Remove excess water and add 100 µl mounting medium to uniformly cover the entire tissue section.

3.2 Place the coverslip without introducing bubbles and wait for the mounting medium to settle.

3.3 Once the coverslip settles, immediately proceed with imaging or store slide laying flat at 4°C in the dark for up to two weeks. If storing multiple slides avoid any contact between slides.
DO NOT exceed two weeks of storage time.

Tissue Imaging

4 **Imaging**

4.1 Image each tissue section individually at the desired magnification using brightfield imaging settings (400x reccomended).

4.2 After imaging, proceed immediately to Coverslip Removal or store as described above.
DO NOT exceed two weeks of storage time.

5 **Coverslip Removal**

5.1 Fill a beaker with 800 ml of Milli-Q water (replace the water after processing 10 slides).

5.2 Submerge the slide holding it parallel to the surface and with the coverslipped surface facing sideways.

5.3 Hold the slide submerged until the coverslip slowly falls off from the slide

5.4 Gently dip the slide 30x in water to remove comletly the mounting medium.

5.5 Let the slide air dry for a minimum of 5 min until the tissue is mostly dry. DO NOT exceed 20 min.

5.6 Incubate slide on the Low Profile Thermocycler Adapter with the thermal cycler lid open for 3 min at 37°C to dry the slide.

5.7 Proceed immediately to Decrosslinking.

Decrosslinking

6 **Destaining**

6.1 Place a Low Profile Thermocycler Adapter in the thermal cycler and set the following parameters.

6.2 Lid Temperature- 42°C
Reaction Volume - 100 µl 15
Run Time - 15 min

	A	B	C
	Step	Temperature	Time
	Pre-equilibrate	42°C	Hold
	Destaining	42°C	00:15:00
	Hold	22°C	Hold

Incubation protocol for thermal cycler

6.3 Place the slide in the Visium CytAssist Tissue Slide Cassette.

6.4 Add 150 µl 0.1 N HCl in a 6.5mm gasket along the side of the wells to uniformly cover the tissue sections (DO NOT introduce bubbles) and ensure uniform coverage.

6.5 Remove HCl by careful aspiration.

6.6 Add 100 µl 0.1 N HCl along the side of the wells to uniformly cover the tissue sections (DO NOT introduce bubbles) and ensure uniform coverage.

6.7 Seal the cassette and place the slide on the Low Profile Thermocycler Adapter at 42°C.

6.8 Close the thermal cycler lid and initiate Destaining.

6.9 Remove the slide from the Low Profile Thermocycler Adapter and place on a flat surface. Some color leftover after the destaining is acceptable.

7 Decrosslinking

7.1 Prepare the thermal cycler with the following settings.

7.2 Lid Temperature- 70°C
Reaction Volume - 100 µl
Run Time - 30 min

	A	B	C
	Step	Temperature	Time
	Pre-equilibrate	70°C	Hold
	Decrosslinking	70°C	00:30:00
	Cooling	22°C	00:10:00
	Hold	22°C	Hold

Incubation protocol for thermal cycler

7.3 Remove all the HCl from the well corners.

7.4 Add 150 µl of diluted decrosslinking buffer.

7.5 Remove diluted decrosslinking buffer.

7.6 Add 100 µl of diluted decrosslinking buffer.

7.7 Re-seal the cassette and place the slide on the Low Profile Thermocycler Adapter at 70°C.

7.8 Close the thermal cycler lid and initiate the decrosslinking.

7.9 Proceed immediately to Visium CytAssist Spatial Gene Expression User Guide (CG000495).