Isolation of Cells from FFPE Tissue Sections for Chromium Fixed RNA Profiling

Introduction

This protocol outlines how to isolate cells from formaldehyde fixed & paraffin embedded (FFPE) Tissue Sections for use with Chromium Fixed RNA Profiling workflow.

The protocol includes instructions for deparaffinizing and rehydrating the tissue sections, followed by preparation and addition of a Dissociation Enzyme Mix to the sections. The tissue sections can then be dissociated using either a pellet pestle or a gentleMACS Octo Dissociator. Once dissociated, cells are resuspended in the Tissue Resuspension Buffer or Quenching Buffer prior to counting.

The minimum recommended cell inputs for Chromium Fixed RNA Profiling hybridization following this protocol are:

- 400,000 cells for singleplex hybridization (Probe Hybridization step 1.1d in User Guide CG000477*)

  When following User Guide CG000477, for steps 2.1g-2.1j, perform one extra 0.5 ml wash for a total of three 0.5 ml post-hybridization washes.

- 100,000 cells per Probe Barcode for multiplex hybridization (Probe Hybridization step 1.1d in User Guide CG000527)

Performing a pilot multiplex assay run (4 rxns with 4 Probe Barcodes) is recommended prior to committing to larger studies.

**Using cells derived from FFPE tissue sections with Feature Barcode technology for cell surface protein detection is not supported.*

For optimal assay results, loading 10,000 cells per Probe Barcode is recommended when performing the singleplex assay or when multiplexing 4 samples. For multiplexing 16 samples, loading 8,000 cells per Probe Barcode is recommended (128,000 is the maximum cells recovery per GEM reaction).

The protocol was demonstrated using FFPE tissue blocks ranging 1-10 years in age. Each block may yield different amounts of material and data quality, depending on age, tissue type, pre-fixation tissue quality, tissue density, size/area of tissue in the scrolls, and other factors.

Additional Guidance

This protocol was demonstrated using two or more 25 µm or 50 µm tissue sections from a diverse spectrum of tissue types. For human tissue, it is recommended to use two or more 25 µm sections and for mouse tissue, it is recommend to use two or more 50 µm sections. Some tissue types require more than two sections to yield enough cells. FFPE block properties will also impact yields. Note that poor quality blocks will likely yield data that cannot be interpreted accurately or salvaged. See Appendix for cell yields derived from tissue sections of indicated tissue types. In rare instances, a single 25 µm or 50 µm section may also yield adequate cells.

Tissue and cells may carry potentially hazardous pathogens. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.

Specific Reagents & Consumables

Vendor	Item	Part Number
For Tissue Section Transfer & Deparaffinization		
Millipore Sigma	Xylene, Reagent Grade	214736
	Ethyl Alcohol, 200 Proof, anhydrous	E7023
VWR	Ethanol absolute ≥99.5%, TechniSolv, pure (for Europe)	83813.360DP
Corning	Phosphate-Buffered Saline, 1X without Calcium and Magnesium	21-040-CV
	15 ml PP Centrifuge Tubes	430791
Thermo Fisher	Nuclease-free Water (not DEPC-Treated)	AM9937
If using gentleMACS Octo Dissociator		
Miltenyi Biotec	gentleMACS C Tubes	130-093-237
Additional Materials		
Disposable plastic Pasteur pipette Nuclease-free Water Water bath Centrifuge		
For Tissue Section Dissociation		
Millipore Sigma	Liberase TH	5401151001
Corning	RPMI	10-040-CV
If using pellet pestle:		
Fisher Scientific	RNase-Free Disposable Pellet Pestles	12-141-364
Fisher Scientific	BD Luer-Lok PrecisionGlide Disposable Syringes with Detachable Needles *OPTIONAL*	14-823-37
If using gentleMACS Octo Dissociator:		
Miltenyi Biotec	gentleMACS Octo Dissociator with Heaters	130-096-427
If using Tissue Resuspension Buffer:		
Millipore Sigma	Protector RNase Inhibitor	3335399001
	Albumin, Bovine Serum*, 10% Aqueous Solution, Nuclease-Free	126615
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin* (BSA, 50 mg/ml)	AM2616
**Choose either Millipore or Thermo Fisher Scientific BSA.*		
VWR	Tris Buffer, 1M sterile sol., pH 8.0	E199-100ML
If using Quenching Buffer:		
10x Genomics	Conc. Quench Buffer**	2000516
***Included in the 10x Genomics Chromium Next GEM Single Cell Fixed RNA Sample Preparation Kit, 16 rxns (PN-1000414); also includes Enhancer (PN-2000482) if storing fixed cells.*		
For Sample Filtration		
Sysmex	Sterile Single-Pack CellTrics Filters***(use 30 µm)	04-004-2326
Miltenyi Biotec	MACS SmartStrainers*** (30 µm)	130-098-458
	OR	
	Pre-Separation Filters***(30 µm)	130-041-407
****Choose either Sysmex or Miltenyi Biotec filter.*		
For Cell Counting		
Nexcelom Biosciences	ViaStain PI Staining Solution	CS2-0109-5mL
	ViaStain AOPI Staining Solution *Alternative to PI Staining Solution*	CS2-0106-5mL
	Cellaca MX High-throughput† Automated Cell Counter	MX-112-0127
Thermo Fisher Scientific	Countess II FL Automated Cell Counter†	AMAQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
	Ethidium Homodimer-1	E1169
†Choose either Countess or Cellaca.		

Refer to SDS and follow local and institutional guidelines for proper handling and disposal of all chemicals.

This list may not include some standard laboratory equipment.

Preparation - Buffers

All buffers should be prepared fresh.

Prepare Dissociation Enzyme Mix, incubate at **37°C** for **10 min** before proceeding with dissociation.

For pestle-based protocol

Dissociation Enzyme Mix	Stock	Final	1 rxn (µl)	4 rxn + 10% (µl)
Liberase TH (mg/ml)	5	1	210	924
RPMI	-	-	840	3696
Total Volume (µl)			**1050**	**4620**

For gentleMACS Octo Dissociator protocol

Dissociation Enzyme Mix	Stock	Final	1 rxn (µl)	4 rxn + 10% (µl)
Liberase TH (mg/ml)	5	1	420	1848
RPMI	-	-	1680	7392
Total Volume (µl)			**2100**	**9240**

Prepare either Tissue Resuspension Buffer or Quenching Buffer. They can be used interchangeably.

Tissue Resuspension Buffer *(Maintain at 4°C)*	Stock	Final	1 rxn (µl)	4 rxn + 10% (µl)
PBS	1X	0.496X	248	1091.2
Tris buffer (pH 8.0; mM)	1000	50	25	110
BSA (RNase free)	10%	0.02%	1	4.4
RNase Inhibitor (U/µl)	40	0.24	3	13.2
Nuclease-free Water	-	-	223	981.2
Total Volume (µl)			**500**	**2200**

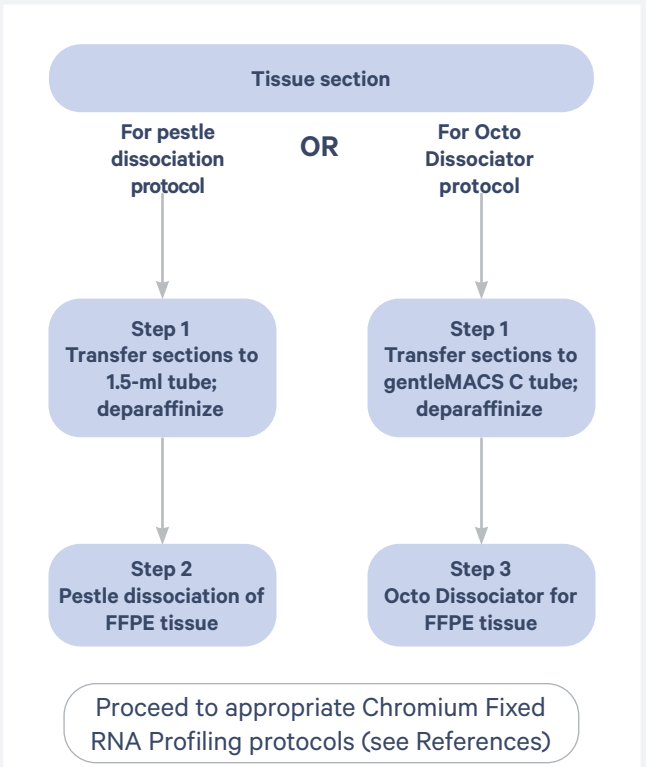
OR

Quenching Buffer *(Maintain at 4°C)*	Stock	Final	1 rxn (µl)	4 rxn + 10% (µl)
Nuclease-free Water	-	-	437.5	1925
Conc. Quench Buffer* (10x Genomics PN 2000516)	8X	1X	62.5	275
Total Volume (µl)			**500**	**2200**

**Included in the 10x Genomics Chromium Next GEM Single Cell Fixed RNA Sample Preparation Kit, 16 rxns (PN-1000414).*

Ethanol: Prepare fresh 70% and 50% Ethanol (1 ml each/sample).

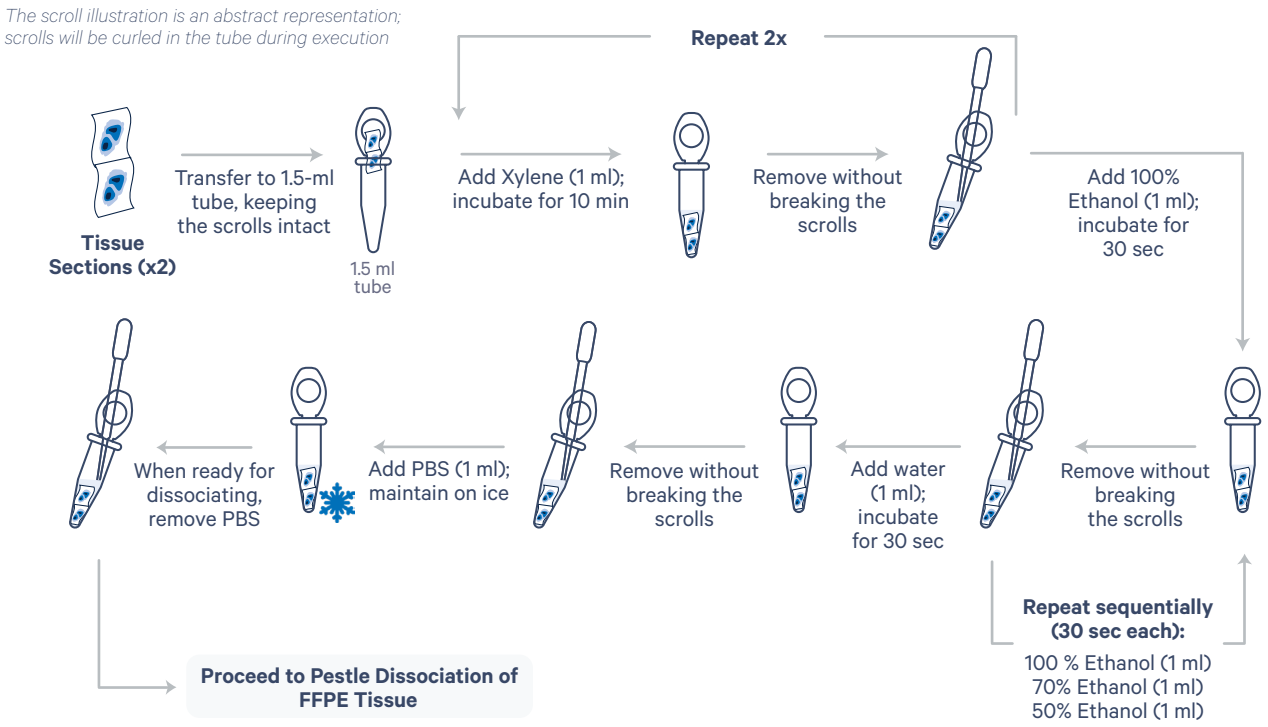
Step Selection Overview



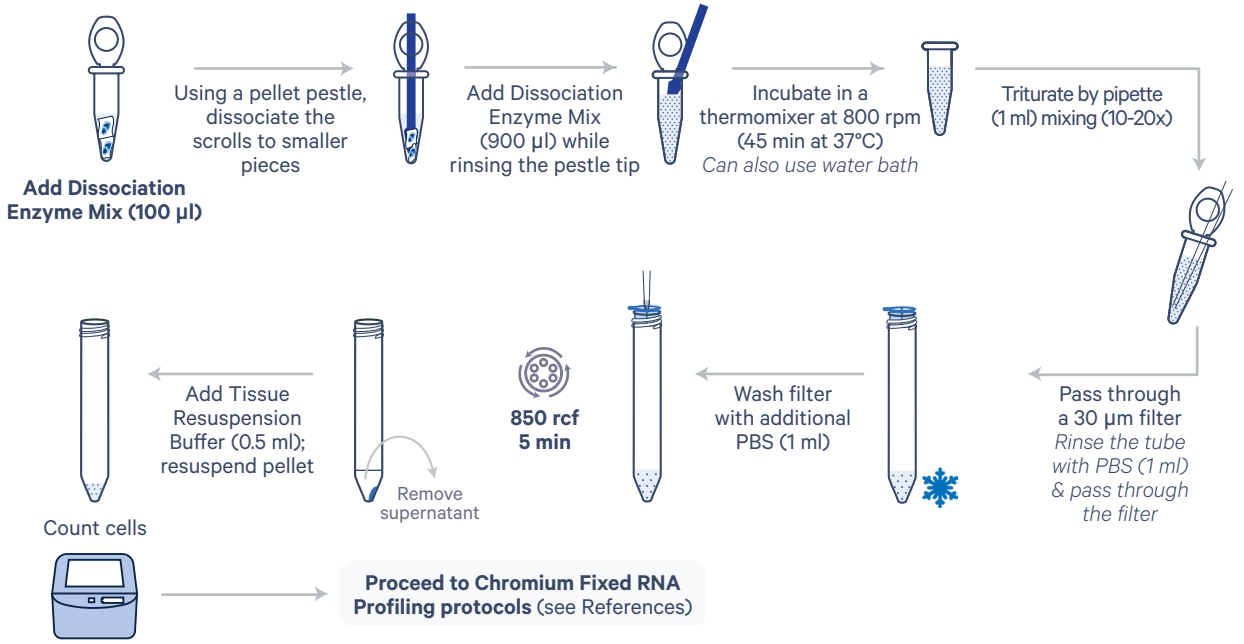
Refer to SDS and follow local and institutional guidelines for proper handling and disposal of all chemicals.

Protocol Overview: Pestle for Isolation of Cells from FFPE Tissue Sections

1. Tissue Section Transfer & Deparaffinization

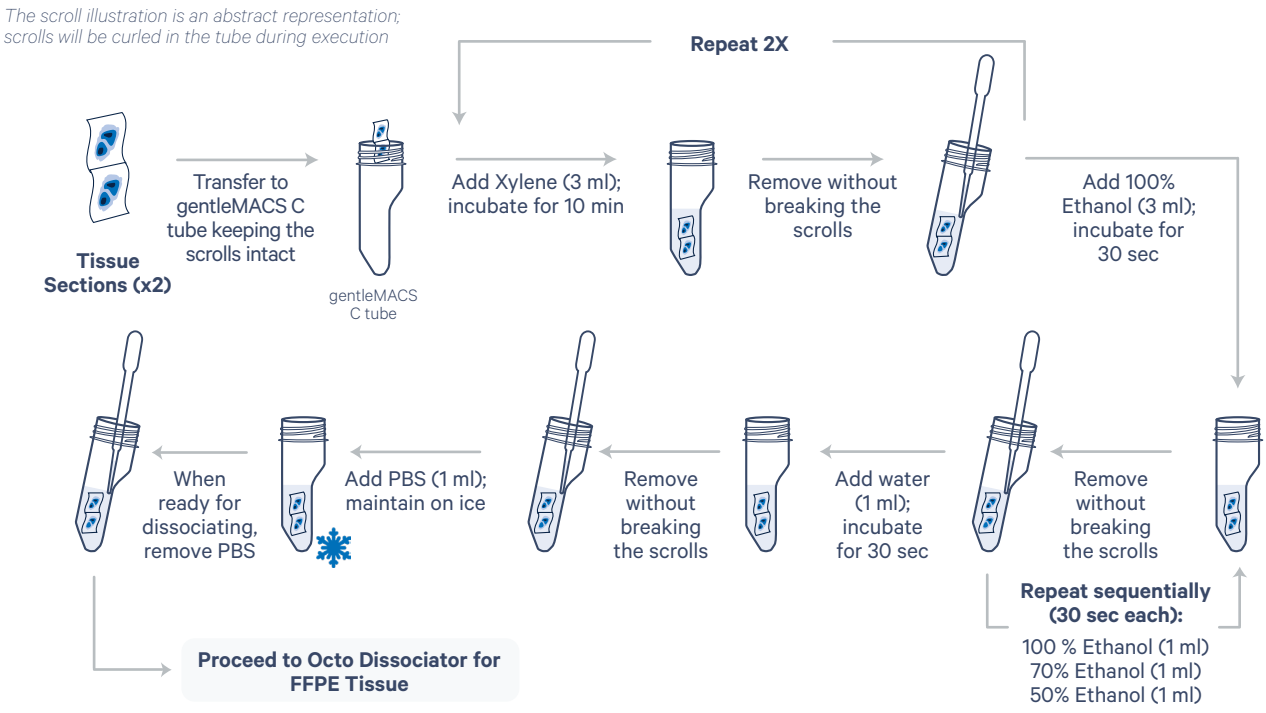


2. Pestle Dissociation of FFPE Tissue

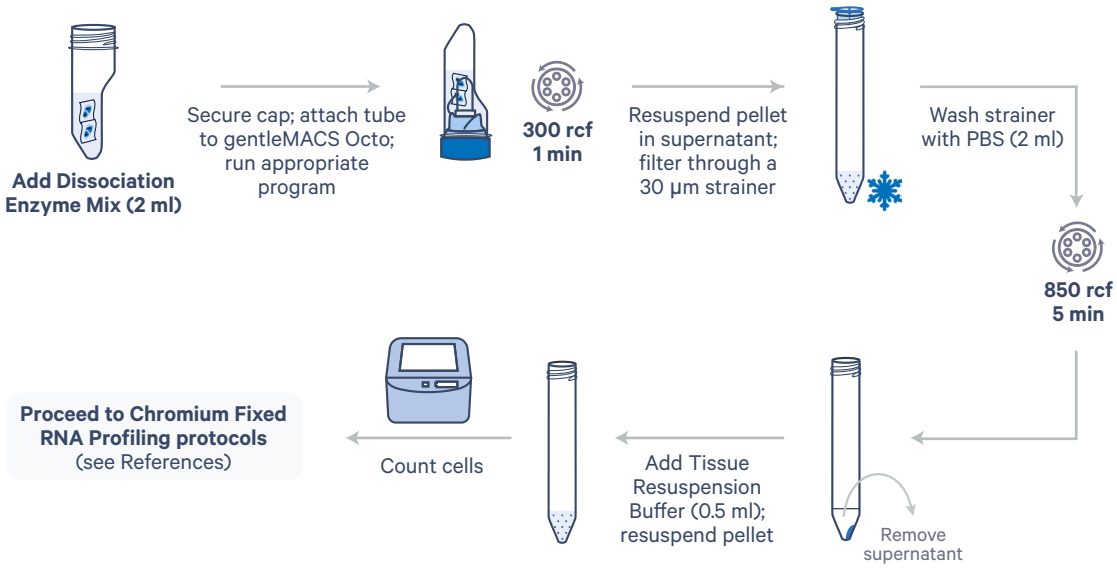


Protocol Overview: gentleMACS Octo Dissociator for Isolation of Cells from FFPE Tissue Sections

1. Tissue Section Transfer & Deparaffinization



3. gentleMACS Octo Dissociator for FFPE Tissue



Isolating Cells from FFPE Tissue Sections

This protocol was demonstrated using 25 µm or 50 µm FFPE tissue sections of various tissue types (see Appendix for details; maximum of six 50 µm sections were dissociated). The cells derived from the FFPE sections are compatible with the Chromium Fixed RNA Profiling assay. The recommended starting cell count for the Chromium Fixed RNA Profiling for the singleplex protocol (CG000477) is 400,000 cells and for the multiplex protocol (CG000527) is 100,000 cells.

1. Tissue Section Transfer & Deparaffinization

Before starting, carefully inspect the tissue block to gauge the extent of dehydration. Face the tissue block and place for sufficient time in an ice water bath (block facing the water surface) to ensure proper rehydration of the tissue block.

See representative images of scrolls derived from well rehydrated and less rehydrated blocks in the Troubleshooting section.

Prepare the first section from the rehydrated tissue block by setting microtome to 5 µm. Discard the first section and set the microtome for 25 µm or 50 µm sections.

The volumes added in steps 1b, 1e, and 1g are based on the type of tube being used for the protocol.

a. After discarding the first section, prepare up to two 25 or two 50 µm sections from the rehydrated tissue block and transfer into a 1.5 ml tube (or to a gentleMACS C Tube) while keeping the scrolls intact. The scrolls in the tube can be stored at **4°C** for up to **one week**.

Ensure that the scrolls stay intact during transfer and through steps 1a-1m. Add reagents along the side of the tube and not directly onto the scrolls. Intact sections enable easier liquid aspiration and minimize tissue loss. If scrolls break, see guidance in Troubleshooting section. Proceed with the protocol and based on cell yields process more sections if needed.

TIPS *Always use fresh/freshly prepared reagents for the deparaffinization steps.*
Ensure the scrolls are fully submerged in regents in the following steps.

b. Add **1 ml** xylene to the 1.5-ml tube (**3 ml** to gentleMACS C Tube) and incubate for **10 min** at **room temperature**.

c. Using a Pasteur pipette, remove the liquid from the tube without breaking the scrolls.

d. Repeat **steps 1b-1c** twice (for a total of three xylene washes).

e. Add **1 ml** 100% ethanol to the 1.5 ml tube (**3 ml** to gentleMACS C Tube) and incubate for **30 sec** at **room temperature.**

f. Using a Pasteur pipette remove the liquid from the tube without breaking the scrolls.

g. Add **1 ml** 100% ethanol (to both tube types) and incubate for **30 sec** at **room temperature.**

h. Using a Pasteur pipette remove the liquid from the tube without breaking the scrolls.

i. Add **1 ml** 70% ethanol (to both tube types) and incubate for **30 sec** at **room temperature.**

j. Using a Pasteur pipette remove the liquid from the tube without breaking the scrolls.

k. Add **1 ml** 50% ethanol (to both tube types) and incubate for **30 sec** at **room temperature.**

l. Using a Pasteur pipette remove the liquid from the tube without breaking the scrolls.

m. Add **1 ml** nuclease-free water to the tube and incubate for **30 sec** at **room temperature**.

n. Using a Pasteur pipette remove the liquid from the tube without breaking the scrolls.

o. Add **1 ml** PBS and maintain on ice.

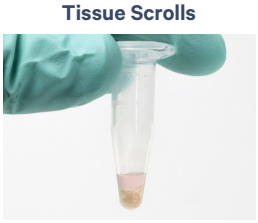
p. Proceed to either to **Step 2 Pestle Dissociation of FFPE Tissue** (sections are in 1.5 ml tube) or to **Step 3 gentleMACS Octo Dissociator for FFPE Tissue** (sections are in gentleMACS C Tube).

2. Pestle Dissociation of FFPE Tissue

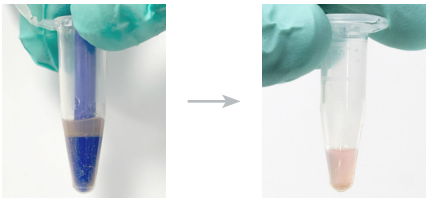
Refer to Preparation - Buffers section to prepare Dissociation Enzyme Mix. Incubate at **37°C** for **10 min** before proceeding with dissociation.

a. Remove the PBS from the tube without breaking the scrolls.

b. Add **100 µl** Dissociation Enzyme Mix to the tube.



c. Using a 1.5 ml pellet pestle, dissociate the tissue scrolls breaking them to smaller pieces.



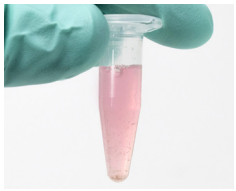
TIPS *Grasp the pestle between the thumb and fingers and rotate the pestle (clockwise and counterclockwise 10-20X) inside the tube and go up and down with the scrolls trapped between the wall of the tube and the pestle for dissociation or until the scroll is broken into similarly sized smaller pieces.*
The pellet pestle can also be attached to a compatible cordless motor and operated inside the tube until the scroll is broken into similarly sized smaller pieces.

d. Add **900 µl** Dissociation Enzyme Mix to the tube while rinsing the pestle tip into the tube to collect any additional tissue pieces sticking to the pestle. Pipette mix.

e. Incubate for **45 min** at **37°C** in a thermomixer at **800 rpm**. Alternatively, incubate in a **37°C** water bath, mixing by inversion every **15 min** during the incubation.

TIPS *Ensure no tissue pieces are stuck to the tube cap. Centrifuge at 300 rcf for ~20 sec to remove pieces stuck on the cap.*

f. Using a 1,000 µl pipette, triturate the tissue pieces in the tube by pipetting 10-20x.



TIPS *The progress of the dissociation can be monitored by taking a 10 µl aliquot and counting. If the cell concentration is lower than recommended for hybridization, additional dissociation maybe required; recommend performing step g.*

g. **OPTIONAL:** Aspirate and push the tissue pieces and solution through a 23G needle 5x to improve cell recovery.

h. Pass the suspension through a Pre-Separation Filter (30 µm) placed on a 5- or 15-ml tube placed on ice.

i. Rinse the original tube (step 2f) with **1 ml** chilled PBS and rinse the 30 µm filter with additional **1 ml** chilled PBS to minimize cell loss. Collect the filtrate in the same tube as step 2h.

j. Centrifuge the cell suspension at **850 rcf** at **4°C** for **5 min**.

TIPS *Use of swinging-bucket rotor is recommended for higher cell recovery.*

k. Remove the supernatant without disturbing the pellet.

l. Resuspend the pellet in **0.5 ml** chilled Tissue Resuspension Buffer or Quenching Buffer, pipette mix 5x, and maintain on ice.

m. Determine cell concentration of the fixed sample using an Automated Cell Counter (Countess II/Cellaca MX) or hemocytometer. See Appendix for Fixed Cell Counting.

TIPS

For accurate cell counting, it is strongly recommended that the cell suspension be stained with a fluorescent nucleic acid dye such as Ethidium Homodimer-1 or PI Staining Solution and counted using an automated fluorescent cell counter.

n. Proceed **immediately** to appropriate Chromium Fixed RNA Profiling protocols – Probe Hybridization step 1.1d (see References) or store according to Fixed Sample Storage Guidance provided in Appendix.

3. gentleMACS Octo Dissociator for FFPE Tissue

Refer to Preparation - Buffers section to prepare Dissociation Enzyme Mix. Incubate at **37°C** for **10 min** before proceeding with dissociation.

a. Remove the PBS from the gentleMACS C Tube without breaking the scrolls.

b. Add **2 ml** Dissociation Enzyme Mix to the gentleMACS C Tube and close securely.

TIPS *Ensure the scrolls are fully submerged in reagents in the following steps.*

c. Place the tube in on the gentleMACS Octo Dissociator , apply Heating units and run the gentleMACS Program 37C_FFPE_1. Run time **~48 min**. Ensure that the Octo Dissociator blades are moving before walking away.

gentleMACS Program 37C_FFPE_1

1	temp ON
2	spin -20 rpm, 5' 0" *(counterclock)*
3	loop 3X
4	spin 20 rpm, 14' 0"
5	spin 1700 rpm, 7"
6	spin 1700 rpm, 1"
7	spin -1700 rpm, 2" *(counterclock)*
8	spin 1700 rpm, 1"
9	spin 1700 rpm, 4"
10	end loop
11	end

d. At the end of the run, detach the tube from the gentleMACS Octo Dissociator and visually inspect to ensure the scrolls have been dissociated. If scrolls are not dissociated, see guidance in Troubleshooting section.

e. Centrifuge at **~300 rcf** for **1 min** and resuspend the cell pellet in the supernatant.

f. Pass the suspension through a Pre-Separation Filter (30 µm) placed on a 15-ml tube placed on ice.

g. Rinse the original gentleMACs tube (step 3d) with **2 ml** chilled PBS and use that rinse for an additional wash of the **30 µm** filter to minimize cell loss. Collect the filtrate in the same tube as step 3f.

TIPS *Use of swinging-bucket rotor is recommended for higher cell recovery.*

h. Centrifuge the cell suspension at **850 rcf** at **4°C** for **5 min**.

i. Remove the supernatant without disturbing the pellet.

j. Resuspend the pellet in **0.5 ml** chilled Tissue Resuspension Buffer or Quenching Buffer, pipette mix 5x, and maintain on ice.

k. Determine cell concentration of the fixed sample using an Automated Cell Counter (Countess II/Cellaca MX) or hemocytometer. See Appendix for Fixed Cell Counting.

TIPS *For accurate cell counting, it is strongly recommended that the cell suspension be stained with a fluorescent nucleic acid dye such as Ethidium Homodimer-1 or PI Staining Solution and counted using an automated fluorescent cell counter.*

l. Proceed **immediately** to appropriate Chromium Fixed RNA Profiling protocols – Probe Hybridization step 1.1d (see References) or store according to Fixed Sample Storage Guidance provided in Appendix.

Appendix

Ethidium Homodimer-1 and PI stained cells can be counted using either Countess II FL Automated Cell Counter or Cellaca Counter.

Counting Using Ethidium Homodimer-1

This protocol provides instructions for counting samples using Ethidium Homodimer-1 and the Countess II FL Automated Cell Counter (with RFP light cube) to enable accurate quantification even in the presence of sub-cellular debris. The optimal cell concentration for the Countess is 1,000-4,000 cells/µl. Refer to manufacturer's instructions for details on operations.

- Vortex Ethidium Homodimer-1, centrifuge briefly, and dilute the concentrated stock as per manufacturer's instructions (~1:100 dilution).
- Aliquot **10 µl** diluted Ethidium Homodimer-1 in a tube.
- Gently mix the sample. Immediately add **10 µl** sample to **10 µl** diluted Ethidium Homodimer-1. Gently pipette mix 10x.
- Transfer **10 µl** sample to a Countess II Cell Counting Slide chamber.
- Insert the slide into the Countess II FL Cell Counter. Image the sample using the RFP setting for fluorescent illumination and filtering. Optimize focus and exposure settings.
- Confirm the absence of large clumps using the bright-field mode. Make sure the cell counter is circling RFP positive cells. Note the RFP-positive concentration. Multiply by dilution factor 2 to determine cell concentration.

Samples stained with Ethidium Homodimer-1 can also be counted using Cellaca Counter. Refer to manufacturer's instructions for details.

Counting using PI Staining Solution

This protocol provides instructions for counting sample using PI staining solution and the Cellaca Counter to enable accurate quantification even in the presence of sub-cellular debris. The optimal cell concentration for the Cellaca Counter is 100-10,000 cells/µl. Refer to manufacturer's instructions for details on operations.

- Add **25 µl** PI Staining Solution into Mixing Row of Cellaca plate
- Gently mix the sample. If the sample is too concentrated, a 1:1 dilution in PBS can also be prepared. For example, add 15 µl fixed cell suspension to 15 µl PBS.
- Add **25 µl** sample to Mixing Row of plate containing PI Staining Solution. Gently pipette mix 8x.
- Transfer stained sample to Loading Row of Cellaca plate.
- For counting fixed samples, only use the PI (Propidium Iodide) channel. Refer to manufacturer's instructions for details.

Samples stained with PI staining solution can also be counted using Countess II FL AutomatedCell Counter. See manufacturer's instructions for details.

When counting cells after dissociation, the suspension may include some debris. However, during the Chromium Fixed RNA Profiling workflow, the post-hybridization wash step will reduce the debris.

Representative images after dissociation and after post-hybridization wash (ready for loading on to the chip for GEM generation) are shown in Figure 1A and 1B respectively.

Figure 1A: Representative Cells after Dissociation

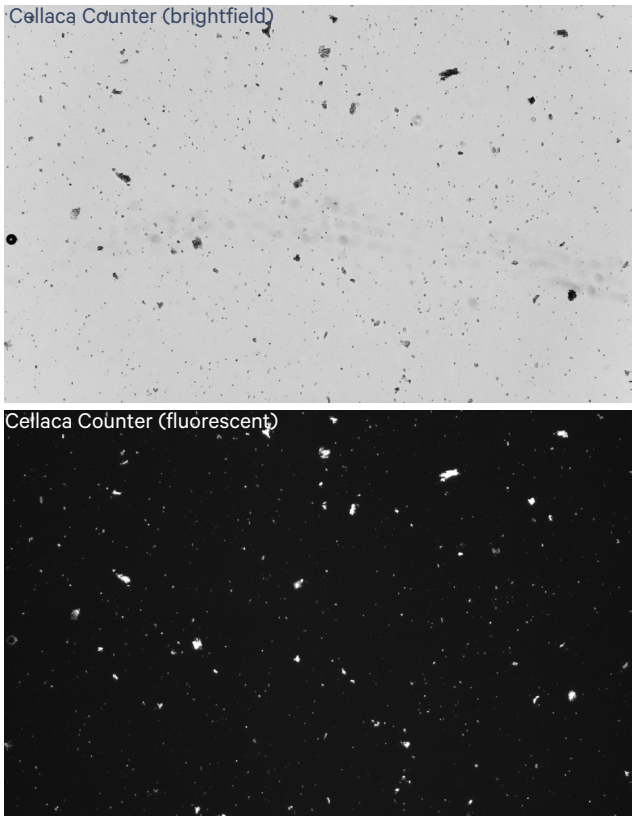
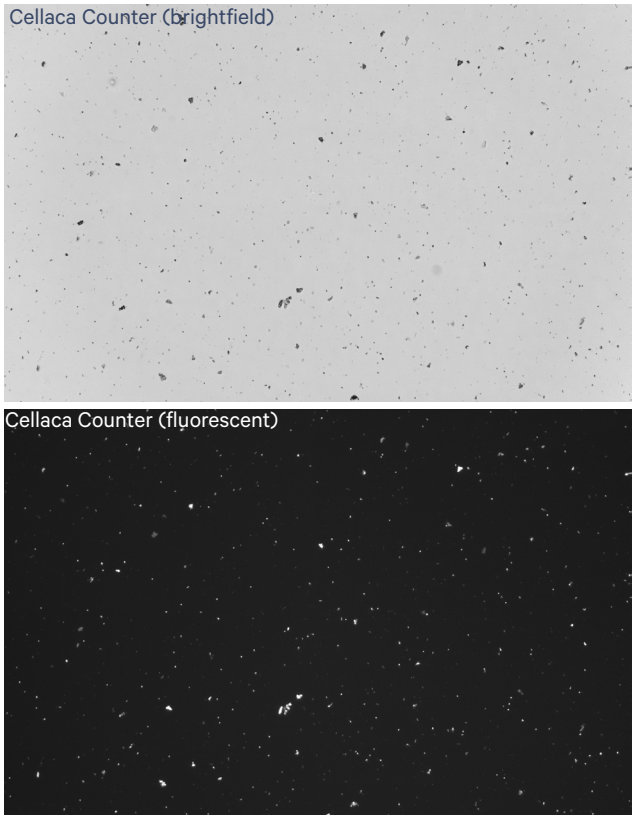


Figure 1B: Representative Cells after Post-Hybridization Wash



Fixed Sample Storage Guidance

Fixed samples (dissociated cells resuspended in Quenching Buffer or in Tissue Resuspension Buffer) can be stored for short or long-term as described below.

Short-term Storage at 4°C

a. Thaw Enhancer (10x Genomics PN-2000482) for **10 min** at **65°C**. Vortex and centrifuge briefly. Keep warm and verify no precipitate before use.

DO NOT keep the thawed reagent on ice, or the solution will precipitate. Once thawed, Enhancer can be kept at 42°C for up to 10 min.

b. Add **0.1 volume** pre-warmed Enhancer to fixed sample in Quenching Buffer or in Tissue Resuspension Buffer. For example, add **50 µl** Enhancer to **500 µl** fixed sample in Quenching Buffer or in Tissue Resuspension Buffer. Pipette mix.

c. Store sample at **4°C** for up to **1 week**.

Long-term Storage at −80°C

a. Thaw Enhancer (10x Genomics PN-2000482) for **10 min** at **65°C**. Vortex and centrifuge briefly. Keep warm and verify no precipitate before use.

DO NOT keep the thawed reagent on ice, or the solution will precipitate. Once thawed, Enhancer can be kept at 42°C for up to 10 min.

b. Add **0.1 volume** pre-warmed Enhancer to fixed sample in Quenching Buffer or in Tissue Resuspension Buffer. For example, add **50 µl** Enhancer to **500 µl** fixed sample in Quenching Buffer or in Tissue Resuspension Buffer. Pipette mix.

c. Add 50% glycerol for a final concentration of 10%. For example: add **137.5 µl** 50% glycerol to **550 µl** fixed sample in Quenching Buffer or in Tissue Resuspension Buffer with Enhancer. Pipette mix.

d. Store at **−80°C** for up to **6 months**.

Post-Storage Processing

Samples may undergo a color change during storage (e.g. black, light gray, or green), however this will not impact assay performance.

If samples were stored at −80°C, thaw at room temperature until no ice is present.

a. Centrifuge sample at **850 rcf** for **5 min** at **room temperature**.

b. Remove the supernatant without disturbing the pellet.

c. Resuspend cell pellet in **0.5 ml** 0.5X PBS + 0.02% BSA (optionally supplemented with 0.2 U/µl RNase Inhibitor) and keep on ice.

Use RNase-free BSA at this step. See Specific Reagents & Consumables for details.

d. Determine cell concentration of the fixed sample using an Automated Cell Counter (Countess II/ Cellaca MX) or hemocytometer. See Counting guidelines.

e. Proceed **immediately** to appropriate Chromium Fixed RNA Profiling protocols (see References).

Cell Yields from FFPE Tissue Sections

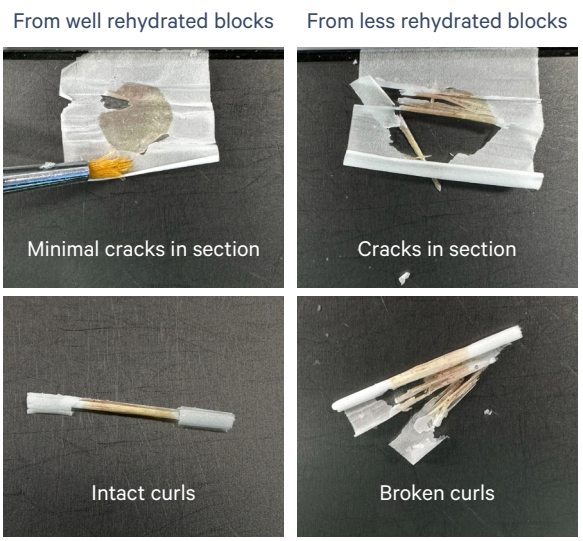
Listed below are the cell yields from FFPE tissue sections processed as described in this protocol. Based on this information, approximate cell yield from a specific tissue type/section may be estimated. Note that the tissue density in combination with tissue cross section area (tissue volume) will impact final cell yields.

	Tissue Type	Tissue State *(Healthy or Diseased)*	No. of Sections x Thickness *(µm)*	Tissue Cross Section *(x*y mm)*	Cell Yields	
					Manual Protocol	gentleMACS Octo Dissociator
MOUSE	Brain (Cerebellum)	Healthy	6 x 50 µm	2 x 2 mm	1.88×10^6	1.24×10^6
	Brain (Forebrain)	Healthy	6 x 50 µm	4 x 5 mm	0.545×10^6	0.2×10^6
	Kidney	Healthy	3 x 50 µm	5 x 9 mm	4.73×10^6	3.89×10^6
	Liver	Healthy	3 x 50 µm	6 x 9 mm	1.7×10^6	1.6×10^6
	Spleen	Healthy	3 x 50 µm	1 x 9 mm	5.15×10^6	4.865×10^6
HUMAN	Brain	Healthy	2 x 25 µm	5 x 10 mm	0.4×10^6	0.36×10^6
	Brain	Glioblastoma	2 x 25 µm	10 x 13 mm	0.77×10^6	0.76×10^6
	Breast	Healthy	2 x 25 µm	10 x 6 mm	0.04×10^6	0.02×10^6
	Breast	Invasive Ductal Carcinoma	2 x 25 µm	13 x 7 mm	1.47×10^6	1.87×10^6
	Cervix	Endocervical Adenocarcinoma	2 x 25 µm	10 x 7 mm	1.2×10^6	1.7×10^6
	Colon	Cancer, Colorectal	2 x 25 µm	10 x 9 mm	0.6×10^6	0.76×10^6
	Kidney	Healthy	2 x 25 µm	7 x 6 mm	0.36×10^6	0.53×10^6
	Liver	Healthy	2 x 25 µm	5 x 6 mm	0.38×10^6	0.61×10^6
	Liver	Hepatocellular Carcinoma	2 x 25 µm	5 x 9 mm	0.56×10^6	0.67×10^6
	Lung	Healthy	2 x 25 µm	9 x 7 mm	0.44×10^6	0.79×10^6
	Lung	Cancer	2 x 25 µm	9 x 8 mm	0.92×10^6	0.76×10^6
	Lymph Node	Healthy	2 x 25 µm	7 x 5 mm	2.34×10^6	2.65×10^6
	Lymph Node	Diseased, Reactive	2 x 25 µm	10 x 12 mm	6.75×10^6	7.45×10^6
	Ovary	Cancer	2 x 25 µm	10 x 18 mm	2.2×10^6	1.92×10^6
	Pancreas	Healthy	2 x 25 µm	11 x 9 mm	0.38×10^6	0.58×10^6
	Prostate	Cancer	2 x 25 µm	9 x 9 mm	0.8×10^6	1.6×10^6
	Skin	Malignant Melanoma	2 x 25 µm	4 x 4 mm	0.22×10^6	0.23×10^6

Troubleshooting

Problem	Solution
Tissue block not adequately rehydrated	Soak in water longer, until rehydrated
Tissue section curl breaks during deparaffinization	Ensure tissue blocks is adequately rehydrated *(see adjacent representative tissue section images from well rehydrated and less rehydrated tissue blocks)* DO NOT pipette directly on top of the curls; pipette against the tube wall
Tissue section curl breaks during rehydration	Centrifuge at 850rcf for 1 min prior to reagent exchange to pellet tissue pieces at the bottom of the tubes
Lower than expected cell yields	Refer to Cell Yields from FFPE Tissue Sections table when planning the experiment If possible, repeat sample preparation protocol with more sections to increase yield Lower cell input recommendation for multiplex hybridization. Consider pooling cells derived from tissues of similar complexity If executing probe hybridization step with lower cell input, ensure that no cells are removed during post-hybridization washes by leaving behind slightly more buffer in the tube during the washes
Debris/large chunks during cell counting	Pass sample through a 30 µm filter followed by rinsing the filter with PBS to minimize cell loss Remaining debris (that passes through the 30 µm filter) will be further reduced during the post-hybridization wash step in the downstream workflow (see Figure 1B)
Using gentleMACS Octo Dissociator	
Intact scroll at the end of run or run fails midway	Run a "spin only" program on the Octo Dissociator with steps 4-9 (from the protocol in step 3C)
Octo Dissociator without heated lids	Use a water bath and every 14 min use the "spin only" program on the Octo Dissociator with steps 4-9 (from the protocol in step 3C)

Representative Tissue Section Images



References

1. Chromium Fixed RNA Profiling Reagent Kit for Singleplexed Samples with Feature Barcode technology for Cell Surface Protein User Guide* (CG000477)
2. Chromium Fixed RNA Profiling Reagent Kit for Multiplexed Samples User Guide (CG000527)

**Using cells derived from FFPE tissue sections with Feature Barcode technology for cell surface protein detection is not supported.*

Document Revision Summary

Document Number	CG000632
Title	Isolation of Cells from FFPE Tissue Sections for Chromium Fixed RNA Profiling
Revision	Rev A
Revision Date	December 2022
Specific Change	Updated long-term fixed sample storage recommendation at −80°C to 6 months (page 12)
General Changes	Updated for general minor consistency of language and terms throughout

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