Tissue Fixation & Dissociation for GEM-X Flex Gene Expression

Introduction

This protocol outlines how to perform tissue fixation followed by dissociation of the fixed tissue for use with GEM-X Flex and Flex v2 workflows. Storage recommendations for the fixed tissue and post-storage processing are listed in the Appendix.

Additional Guidance

This protocol was demonstrated using 25 mg of various mouse and human tissue types (flash-frozen and fresh), including brain, heart, liver, spleen, testes, ileum, duodenum, pancreas, kidney, lung, colon, tonsil, lymph, etc. See Appendix for a complete list of tissues tested along with corresponding cell yields. Optimize the protocol based on tissue type, tissue density, and dissociation efficiency. For the latest information on various tissue types tested, see the 10x Genomics support website. Certain tissue samples like pancreas are challenging to work with and may lead to variable data quality.

Across a limited set of sample types, 10x Genomics found that Liberase TH often led to more efficient dissociation and higher sensitivity. If desired, consider testing Liberase TL vs Liberase TH at 0.2-1 mg/ml concentration for a given tissue of interest.

Tissue and cells 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 Mincing & Fixation		
10x Genomics	GEM-X Flex Sample Preparation v2 Kit	1000781
Thermo Fisher Scientific	Formaldehyde (37% by Weight/ Molecular Biology), Fisher BioReagents	BP531-25
	Nuclease-free Water (not DEPC-Treated)	AM9937
	Pyrex Petri Dish*	08-748D
	General-Purpose Forceps*	10-270
	Standard Dissecting Scissors*	08-951-20
	Wide-Bore Pipette Tips RT LTS 1000 uL*	FLW 768A/8 (30389218)
	**May be procured from alternative vendors*	
Corning	Phosphate-Buffered Saline, 1X *without Calcium and Magnesium*	21-040-CV
Additional Materials		
Blade; Lab Weighing Scale		
This list may not include some standard laboratory equipment.		
For Tissue Dissociation		
Millipore Sigma	Liberase TH Liberase TL *(alternative to TH)*	5401135001 5401020001
Miltenyi Biotec	gentleMACS Octo Dissociator with Heaters gentleMACS C Tubes	130-096-427 130-093-237
Corning	Corning RPMI 1640	10-040-CV

Thermo Fisher Scientific	Gibco RPMI 1640	11875093
Choose either Corning or Gibco RPMI		
For Sample Filtration *Choose one based on availability or preference*		
Sysmex	Sterile Single-Pack CellTrics Filters (30 µm)	04-004-2326
Miltenyi Biotec	MACS SmartStrainers (30 µm)	130-098-458
	OR Pre-Separation Filters (30 µm)	130-041-407
Thermo Fisher Scientific	Pre-Separation Filters (30 µm)	43-10040
For Cell Counting		
Nexcelom Biosciences	ViaStain PI Staining Solution	CS1-0109-5mL
	ViaStain AOPI Staining Solution *Alternative to PI Staining Solution.*	CS2-0106-5mL
	†Cellaca MX High-throughput Automated Cell Counter	MX-112-0127
	†Cellometer K2 Fluorescent Cell Counter	CMT-K2-MX-150
	PD100 Counting Chambers 1 case	CHT4-PD100-003
Biotium	NucSpot 470 *Alternative to PI Staining Solution.* *Dilute the stock to 1:100 and mix 1:1 with the sample. For example, add 10 µl diluted dye to 10 µl sample.*	40083
Thermo Fisher Scientific	†Countess II FL Automated Cell Counter *Discontinued*	AMAQAF1000
	Countess Automated Cell Counting Chamber Slides	C10228
	†Countess 3 FL Automated Cell Counter	AMQAF2000
	Trypan Blue Stain (0.4%)	T10282
†Choose Countess II/3, Cellaca, Cellometer, or equivalent fluorescent counter.		
For Storage & Post-Storage Processing		
Acros Organics	Glycerol, 99.5%, for molecular biology, DNAse, RNAse and Protease free *Alternative to Millipore Sigma product*	327255000
Millipore Sigma	Glycerol for molecular biology, ≥99.0% *Alternative to Acros Organics product*	G5516-100ML
VWR	Vacuum Filter/Storage System (0.2 µm)	29442-936
Additional Materials		
Eppendorf	DNA LoBind Tubes 2.0 ml	022431048
	DNA LoBind Tubes 5.0 ml	0030108310
	ThermoMixer C	5382000023
Corning	15-ml Centrifuge Tube	430790

GEM-X Flex Sample Preparation v2 Kit PN-1000781

GEM-X Flex Sample Preparation v2 Kit
PN-1000781
Shipped on dry ice
Store at -20°C

	#	PN
Conc. Fix & Perm Buffer B	2	2001301
Conc. Quench Buffer B	6	2001300
Enhancer	3	2000482
Additive C***	4	2001332

10x GENOMICS

****Not used in this protocol.*

The sample preparation kit provides sufficient reagents to process 24 tissue samples, each weighing 25 mg. If the tissue mass exceeds 25 mg, the kit will support fewer than 24 reactions to account for the scaled up buffer volumes needed.

Transnetyx	Fire Polished Pipette	FPP

This list may not include some standard laboratory equipment.

Preparation - Buffers

All buffer preparations should be fresh.

Buffers for Fixation - Prepare fresh (1 ml)			
Fixation Buffer B Maintain at room temperature	**Stock**	**Final**	**Per 25 mg tissue (µl) + 10%**
Nuclease-free Water	-	-	870
Conc. Fix & Perm Buffer B* (10x Genomics PN 2001301) *Thaw at room temperature. Vortex, check for precipitation, and centrifuge briefly. Maintain at room temperature. If precipitate is observed, heat at 42°C for 10 min.*	10X	1X	110
Formaldehyde	37%	4%	120

Formaldehyde should always be used with adequate ventilation, preferably in a fume hood. Follow appropriate regulations.

Quenching Buffer B** Maintain at 4°C	**Stock**	**Final**	**Per sample (µl)**
Nuclease-free Water	-	-	875.0
Conc. Quench Buffer B* (10x Genomics PN 2001300) *Thaw at room temperature. Vortex and centrifuge briefly. Maintain at 4°C*	8X	1X	125.0

**Included in the 10x Genomics GEM-X Flex Sample Preparation v2 Kit, (PN-1000781).*

***Total 2 ml Quenching Buffer B will be needed if immediately proceeding to dissociation after fixation. If planning to store the fixed tissue pieces, 1 ml buffer will be sufficient.*

Dissociation Solution Prepare 2 ml

- Prepare Liberase TH stock solution (5 mg/ml) by adding 1 ml nuclease-free water to 5 mg Liberase TH. Mix at 2-8°C until completely resuspended. Store in single-use aliquots at -20°C.
- Prepare RPMI + 1 mg/ml Liberase *(Add 420 µl Liberase stock solution into 1,680 µl of RPMI, mix, maintain at 37°C until use).*

Buffers for Storage of Fixed Samples - Prepare fresh

50% Glycerol Solution
(needed for long-term storage of fixed samples)

Tips & Best Practices

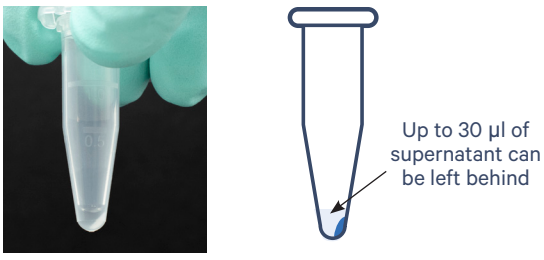
The following recommendations are critical for optimal performance of the GEM-X Flex and Flex v2 assays.

Tissue Quality & Processing

- Perform pilot experiments to determine if the tissue type is suitable for fixation and dissociation for preparing single cell suspension(s).
- Ensure that variation in cell yields from different tissue types is considered when performing the protocol.
- The tissue should be minced only on a glass surface. DO NOT use plastic petri dish for mincing tissue.

Centrifugation & Pellet Resuspension

- Use a swinging-bucket rotor for higher cell/nuclei recovery.
- Centrifugation speed and time may need optimization depending upon the sample type.
- When working with samples with low cell numbers (i.e. <300,000 cells), complete removal of the supernatant is not required. Up to 30 µl supernatant may be left behind to optimize cell recovery without significantly impacting assay performance.
- After each buffer addition step, gently mix cells/nuclei 5x, or until the pellet is completely resuspended, without introducing bubbles.



Fixed Cell Counting

- Samples should have minimal debris for best results; debris can have associated RNA that can contribute to non-cell background.
- Accurate sample counting is critical for achieving desired cell recovery.
- Counting fixed samples in Quenching Buffer B before hybridization is recommended if samples will be multiplexed and the amounts of cells/nuclei added into each hybridization need to be normalized. Counting is also encouraged if unsure of low input sample amount.
- It is strongly recommended that the sample be stained with a fluorescent nucleic acid dye such as AO/PI Staining Solution and counted using an automated fluorescent cell counter or hemocytometer.
- See Appendix for details on fixed cell counting.

Fixed Sample Storage

- Fixed tissue pieces and cell suspension derived from fixed dissociated tissues can be stored at 4°C for up to 1 week or at -80°C for up to 12 months after resuspending in appropriate reagents.
- Sample storage guidelines are provided in the Appendix.

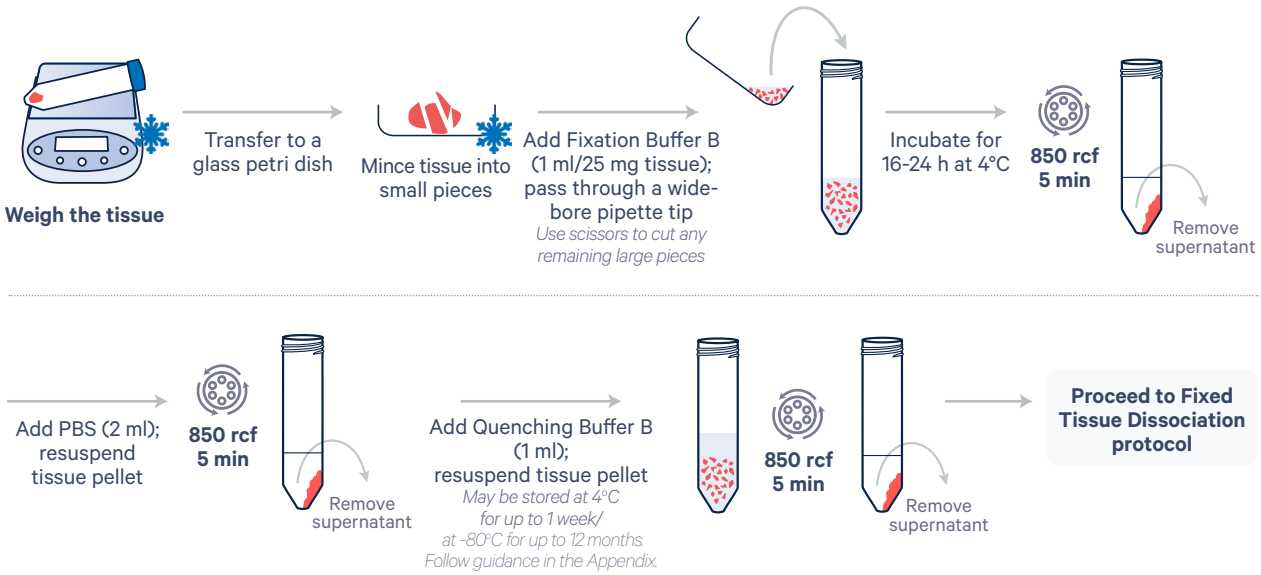
Cell/Nuclei Number Recommendation

- Some cell loss is expected during the fixation steps depending on the sample type, cell type, and user experience.

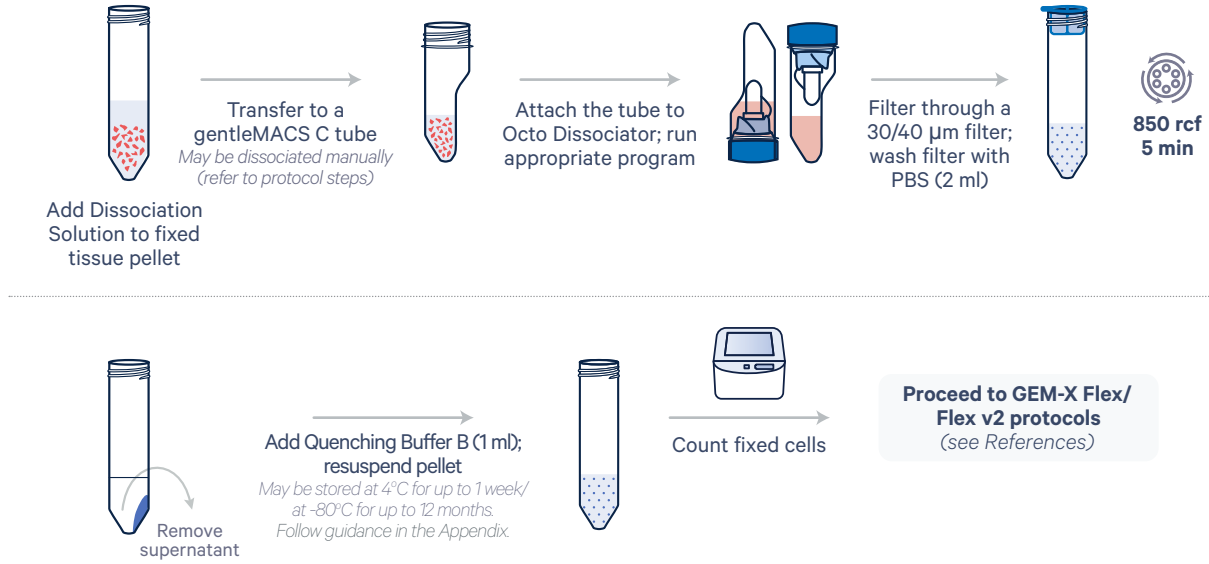
Recommended Cell/Nuclei Input per Hybridization
25,000–500,000 *(if sufficient cells are available, it is recommended to default to 300,000 fixed cells)*
Important Considerations • See the Cell Yields from Fixed Dissociated Tissues table in the Appendix for additional tissue-specific input recommendations. It may be possible to use ≤25,000 cells/nuclei, but it may lead to: • Loss of pellet • Not enough cells for storage • Difficulty in pooling samples in equal number when multiplexing • Not enough cells left after washing to target maximum cell load (20,000 cells/Probe Barcode) • Difficulty in counting samples; may require concentrating the sample • Observance of an atypical library trace and increase in wasted data due to excess supernatant left behind during post hybridization washes
Mitigation Strategies • Follow better sample preparation practices including use of a swinging bucket rotor • During probe hybridization, up to 15 µl supernatant can be left behind to avoid losing the pellet • During post-hybridization wash, up to 30 µl supernatant can be left behind to avoid losing the pellet • Follow pooled wash workflow during post-hybridization wash

Protocol Overview : Tissue Fixation & Dissociation

1. Tissue Fixation



2. Fixed Tissue Dissociation



Tissue Fixation & Dissociation Protocol

This protocol was demonstrated using 25 mg fresh or flash frozen tissue. For most tissues, less than 25 mg may also yield sufficient cell numbers. See Appendix for a complete list of tissues tested along with additional guidance for certain tissue types as well as corresponding cell yields from 25 mg of tissue. GEM-X Flex Sample Preparation v2 Kit (PN-1000781) was used for tissue fixation.

1. Fix Tissue

If using frozen tissue, maintain the tissue on dry ice before and after weighing. Use a pre-chilled glass petri dish placed on ice while mincing the tissue.

a. Weigh tissue to determine Fixation Buffer B volume. 1 ml Fixation Buffer/25 mg tissue will be used at step 1c. Prepare Fixation Buffer before proceeding to next step.

b. Place the tissue on a pre-chilled **glass** petri dish maintained on ice and using a blade, mince tissue finely (enables passing through a 1 ml wide-bore pipette tip).

c. Using a **wide-bore** (1.5 mm) 1-ml pipette, add **1 ml** Fixation Buffer B/25 mg tissue (see table below). Pass the tissue up and down. Aspirate the solution containing the minced tissue and transfer to a 2-ml or larger centrifuge tube. Pass the tissue up and down. If larger tissue pieces fail to pass through the pipette, use dissection scissors to cut the tissue pieces further until they pass through the pipette. Maintain on **ice**.

Tissue Mass (mg)	Fixation Buffer B (ml)
<37 mg	1 ml
38-62 mg	2 ml
63-87 mg	3 ml
88-112 mg	4 ml

d. Incubate for **16-24 h** at **4°C**.

DO NOT agitate or mix the sample during incubation.

Fixation time and temperature should be consistent across all samples in an experiment.

If working with spleen, incubate for 16-24 h at room temperature (20°C).

e. Centrifuge at **850 rcf** for **5 min** at **4°C**.

f. Remove the supernatant without disturbing the tissue pellet.

g. Add **2 ml** chilled PBS and resuspend the tissue pellet.

h. Centrifuge at **850 rcf** for **5 min** at **room temperature**.

i. Remove the supernatant without disturbing the tissue pellet.

j. Add **1 ml** Quenching Buffer B, resuspend the tissue pellet, and maintain on ice.

STOP

Fixed tissue pieces can be stored at 4°C for up to 1 week or at -80°C for up to 12 months. See Appendix for guidance on short-term storage and post-storage processing of fixed tissue pieces.

k. Centrifuge at **850 rcf** for **5 min** at **room temperature**.

l. Remove the supernatant without disturbing the fixed tissue pellet.

m. Proceed to tissue dissociation.

2. Dissociate Fixed Tissue

a. Warm Dissociation Solution for **10 min** at **37°C** before use.

b. Add **2 ml** pre-warmed Dissociation Solution to the sample.

Composition of Dissociation Solution should be consistent across all samples in an experiment. Use 2 ml Dissociation Solution for up to 100 mg tissue. Use only 2 ml Dissociation Solution per C tube.

c. Dissociate tissue. Using an Octo Dissociator is highly recommended. Alternatively, tissue may be dissociated manually.

- **Octo Dissociator:**
  Transfer to Miltenyi C tubes. Use multiple Miltenyi C tubes if tissue exceeds 100 mg and use 2-ml Dissociation Solution/C tube. Ensure that no fixed tissue pieces are stuck on the walls of the C tube. Place the C tube in the Octo Dissociator, apply Heating Units, and run the following program:

gentleMACS Program	
1	temp ON
2	spin 50 rpm, 20' 0"
3	spin 1000 rpm, 30"
4	spin -1000 rpm, 30" *(counterclock)*
5	end

Dissociation can be monitored by taking a 10 µl aliquot for counting. If the cell concentration is lower than expected, additional dissociation may be required. See Troubleshooting guidance.

- Detach the C tubes and proceed to step 2d. **OPTIONAL:** Centrifuge the C tubes at **300 rcf** briefly to collect all the cells at the bottom of the tube. Resuspend the pellet in the supernatant and proceed to step 2d.

OR

- **Manual Dissociation:**
  Incubate for **20 min** at **37°C** shaking the tube intermittently. Using a P1000 regular-bore pipette tip or a silanized glass pipette, triturate the tissue pieces 15-20X (until solution begins to turn cloudy) to obtain a single cell suspension. Proceed to step 2d.

If tissue is tough to triturate and/or solution is not cloudy after trituration, see Troubleshooting guidance

d. Pass the dissociated tissue through a 30 or 40 µm filter to remove debris and undissociated tissue pieces and collect in 15-ml centrifuge tube or 5-ml LoBind tube.

e. Perform an additional wash of the 30 or 40 µm filter by adding **2 ml** PBS to the filter. Collect the filtrate in the same tube as step 2d.

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

f. Centrifuge at **850 rcf** for **5 min**.

g. Remove the supernatant without disturbing the pellet.

h. Resuspend pellet in **1 ml** chilled Quenching Buffer B.

i. Determine the cell concentration using a Countess II/3 FL Automated Cell Counter or hemocytometer. See Appendix for Fixed Cell Counting.

TIPS *For accurate cell counting, it is strongly recommended that the cell/nuclei suspension be stained with a fluorescent nucleic acid dye such as AO/PI Staining Solution and counted using an automated fluorescent cell counter.*
If the cell suspension contains aggregates or debris larger than 30 µm, pass through a 30 or 40 µm filter and recount.

For lower than expected cell yields, see Troubleshooting guidance.

j. Proceed **immediately** to appropriate GEM-X Flex/Flex v2 protocols (see References) or store the sample after resuspending in appropriate reagents.

STOP *Samples can be stored at 4°C for up to 1 week or at -80°C for up to 12 months, depending upon the buffers used. See Appendix for guidance on short and long-term storage and post-storage processing of fixed tissue pieces and cells.*

Appendix

Fixed Sample Storage Guidance

Fixed samples can be stored for short or long-term.

Short-term Storage at 4°C (for fixed tissue pieces & cells)

a. Thaw Enhancer 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. If not used within 10 min, reheat at 65°C to ensure enhancer is fully dissolved..

b. Add **0.1 volume** pre-warmed Enhancer to fixed tissue pieces or cells in Quenching Buffer B. For example, add 100 µl Enhancer to 1,000 µl fixed cells in Quenching Buffer B. Pipette mix.

Alternatively, to conserve the Enhancer volume, centrifuge cells at 850 rcf, remove 500 µl Quenching Buffer B, and add 50 µl Enhancer to the sample.

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

Long-term Storage at −80°C (for fixed tissue pieces & cells)

a. Thaw Enhancer 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. If not used within 10 min, reheat at 65°C to ensure enhancer is fully dissolved.

b. Add **0.1 volume** of pre-warmed Enhancer to fixed sample in Quenching Buffer B. For example, add 100 µl Enhancer to 1,000 µl fixed sample in Quenching Buffer B. Pipette mix.

Alternatively, to conserve the Enhancer volume, centrifuge cells at 850 rcf, remove 500 µl Quenching Buffer B, and add 50 µl Enhancer to the sample.

c. Add 50% Glycerol for a final concentration of 10%. For example: add **275 µl** 50% Glycerol to **1,100 µl** fixed sample in Quenching Buffer B and Enhancer. Pipette mix.

d. Store at **−80°C** for up to **12 months**.

TIPS *Storing fixed cells at −80°C is recommended for best results.*

Fixed Tissue Pieces – 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 tissue pellet.

c. Proceed to Dissociate Fixed Tissue protocol (step 2a).

Fixed Cells – 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 1 ml Quenching Buffer B and maintain on ice.

d. Determine cell concentration of the fixed sample using an automated cell counter or hemocytometer. See Appendix for Fixed Cell Counting.

e. Proceed **immediately** to appropriate GEM-X Flex/Flex v2 protocols (see References).

Fixed Sample Shipping Guidance

a. Fixed tissue pieces or single cells resuspended in Quenching Buffer B supplemented with Enhancer can be shipped with a cold pack. See Short-term Storage for details.

b. Fixed single cells or fixed tissue pieces resuspended in Quenching Buffer B supplemented with Enhancer and Glycerol can be shipped on dry ice. See Long-term Storage for details.

Fixed Cell/Nuclei Counting

- Accurate sample counting is critical achieving desired cell recovery. The table below shows the combination of counters and dyes tested for counting nuclei post-hybridization and post-hybridization wash. This information also applies to the counting steps in this Demonstrated Protocol.
- It is strongly recommended that the fixed sample be stained with a fluorescent nucleic acid dye such AO/PI staining solution and counted using an automated fluorescent cell counter or hemocytometer.
- The use of fluorescent dye during cell counting enables accurate quantification even in the presence of sub-cellular debris.
- Automated fluorescent cell counters are strongly recommended when counting fixed cells.
- Ensure that the counter excitation/emission filter setup is compatible with the fluorescent dye used.
- Ensure cells are well-focused under brightfield before switching to the fluorescent channel for counting.
- Increase exposure time to help adjust signal to noise during counting.
- Perform visual inspection to confirm that the counting number is accurate. For example, after obtaining counts, switch from the brightfield channel to the fluorescent channel to ensure that the counts primarily include cells with minimal debris.

Counting using AO/PI Staining Solution

This protocol provides instructions for counting sample using AO/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** AO/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 AO/PI Staining Solution. Gently pipette mix 8x.
- Transfer stained sample to Loading Row of Cellaca plate.

Counter Type	Fluorescent Dye	Counting Comparison
Cellaca *Range: 1 x 10^5–1 x 10^7cells/ml* *Automated exclusion of debris from cell count*	• Propidium Iodide • NucSpot 470 • DAPI	Comparable counting results at both counting steps for all three dyes
Countess II FL/Countess 3 FL *Range: 1 x 10^4–1 x 10^7cells/ml (optimal 1 x 10^5–4 x 10^6)* *Manual debris exclusion from cell count post-image capture, using gates on the instrument program*	• Propidium Iodide • NucSpot 470 • DAPI	Comparable counting results at both counting steps for the three dyes
Cellometer K2 *Range: 1 x 10^5–1 x 10^7cells/ml* *Debris exclusion from cell count by adjusting instrument program settings before image capture*	• Propidium Iodide • NucSpot 470	Comparable counting results at both counting steps for the two dyes Propidium iodide stained cells/nuclei are relatively dimmer and require longer exposure than NucSpot 470, so NucSpot 470 is preferred

Cell Yields from Fixed Dissociated Tissues

Listed below are the cell yields from 25 mg of various mouse and human tissue types (flash-frozen or fresh) that were processed with 0.2 mg/ml Liberase TL. Using Liberase TH will result in similar/slightly higher cell yields. Refer to the tested tissues listed on the 10x Genomics support website for additional important considerations. The final cell yields are impacted by the cell density and morphology of the tissue, and by the overall tissue quality prior to fixation. Less than 25 mg of some of the listed tissues may also yield sufficient cells. For example, based on this table, 2 mg of flash-frozen human lung is expected to yield ~ 272,000 cells.

	Tissue Type	Cell Yields/25 mg tissue Flash-frozen tissue
Human	Jejunum	1.37 x 10^6
	Colon	0.04 x 10^6 *Lower than expected yield from 25 mg colon tissue; using >25 mg recommended for this assay*
	Lung	3.4 x 106
	Kidney	2 x 10^6
	Liver	1.8 x 10^6
	Spleen	9.6 x 10^6 *If working with spleen, fix at room temperature 16-24 h. The max recommended cell input into hybridization is 100,000.*
	Ileum	0.14 x 10^6
	Testes	2.05 x 10^6
	Duodenum	0.32 x 10^6
	Breast Cancer	0.96 x 10^6
	Melanoma	3.18 x 10^6
	Ovarian Cancer	0.93 x 10^6
	Pancreatic Cancer	1.78 x 10^6 cells *The maximum recommended cell input into hybridization is 100,000.*

	Tissue Type	Cell Yields/25 mg tissue	
		Flash-frozen tissue	Fresh tissue
Mouse	Adipose	Not recommended *Nuclei isolation & fixation recommended*	
	Bladder	0.7 x 10^6	Not tested
	Brain (Forebrain)	1 x 10^6	0.79 x 10^6
	Brain (Cerebellum)	Not tested	3.1 x 10^6
	Brain (Whole)	1.6 x 10^6	0.84 x 10^6
	Colon	2.2 x 10^6	Not tested
	Eye	1.6 x 10^6	Not tested
	Heart	0.99 x 10^6	2 x 10^6
	Intestine	3.1 x 10^6	1.6 x 10^6
	Kidney	2.7 x 10^6	2.5 x 10^6
	Liver	1.3 x 10^6	0.57 x 10^6
	Lung	2 x 10^6	3.1 x 10^6
	Muscle	0.41 x 10^6	Not tested
	Ovary	3.2 x 10^6	Not tested
	Pancreas	1.8 x 10^6 *The maximum recommended cell input into hybridization is 100,000.*	0.92 x 10^6
	Spinal Cord	1.5 x 10^6 *High debris observed*	Not tested
	Spleen	14 x 10^6 *If working with spleen, fix at room temperature 16-24 h. The max recommended cell input into hybridization is 100,000.*	6.3 x 10^6
	Stomach	2.5 x 10^6	Not tested
	Testis	4.2 x 10^6	Not tested
	Thymus	15 x 10^6	26 x 10^6
	Tongue	1.4 x 10^6	Not tested

Troubleshooting

Problem	Solution
Lower than expected cell yield after dissociation	• Increase the tissue dissociation time. • If using an Octo Dissociator, after transferring the dissociated cells from the C tube, perform an additional PBS rinse of the C tube and pass the rinse through the 30 or 40 µm strainer in step 2e to collect additional cells. • If using gentleMACS Octo Dissociator, intact/large tissue pieces at the end of run or run fails midway. Run a "spin only" program on the Octo Dissociator with steps 3-4 (from the protocol in step 2C). • If manually dissociating tough tissue, use the back of a 1-ml syringe plunger to push any undissociated tissue pieces through the 30 or 40 µm strainer.

References

GEM-X Flex v2

1. GEM-X Flex v2 - Protocol Planner (CG000832)
2. GEM-X Flex v2 User Guide (CG000834)
3. GEM-X Flex v2 for Singleplexed Samples with Feature Barcode technology for Protein (CG000841)

GEM-X Flex

1. GEM-X Flex Gene Expression - Protocol Planner (CG000780)
2. GEM-X Flex Gene Expression Reagent Kit for Singleplexed Samples User Guide (CG000786)
3. GEM-X Flex Gene Expression Reagent Kit for Multiplexed Samples User Guide (CG000787)

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Document Revision Summary

Document Number	CG000783
Title	Tissue Fixation & Dissociation for GEM-X Flex Gene Expression
Revision	Rev B to Rev C
Revision Date	October 2025
Description of Changes	Updated for general minor consistency of language, format, and terms throughout Updated to indicate compatibility with GEM-X Flex v2 along with references on pages 1 and 12 Added a note about number of samples that can be processed using the sample preparation kit on page 2 Updated notes about Enhancer handling on page 9

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