

Plate-based Sample Fixation for GEM-X Flex v2

Introduction

GEM-X Flex v2 offers comprehensive scalable solutions to measure gene expression in up to 384 formaldehyde fixed samples to be run within a single GEM reaction. This protocol outlines sample fixation and quenching in a 96-well V-bottom deep-well plate to enable high throughput handling of up to 96 samples (one 96-well plate) or 384 samples (four 96-well plates) for use with the GEM-X Flex v2 workflow. It also provides guidance on best practices for sample handling and storage and post-storage processing.

Additional Guidance

This protocol works with both fresh cell/nuclei suspension and samples labeled with an antibody conjugated to a Feature Barcode oligonucleotide. For details on sample labeling, see the Demonstrated Protocol Cell Surface & Intracellular Protein Labeling for GEM-X Flex Gene Expression (CG000781).

- Consult the Handbook – Cell Preparation Guide (CG000053) for Tips & Best Practices during sample preparation and for more information on determining accurate cell counts.
- Consult GEM-X Flex v2 - Protocol Planner (CG000832) for details on workflow overview, document resources, and guidance on selecting the appropriate sample preparation and library construction protocols for different GEM-X Flex v2 workflows.
- After the completion of fixation and quenching steps described in this document, proceed to the appropriate GEM-X Flex v2 User Guide (CG000834 or CG000835). Preread the User Guides for additional tips and best practices on multiplexing.

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.

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Reagents & Consumables

10x reagents are listed in the same order as their placement in the kits.

Refer to SDS for handling and disposal information.

GEM-X Flex Sample Preparation v2 Kit PN-1000781

GEM-X Flex Sample Preparation v2 Kit – Module 1					
PN-1000781					
Shipped on dry ice; Store at -20°C					
Cap Color	Reagent	PN	Cap Color	Reagent	PN
●	Conc. Fix & Perm Buffer B	2001301	●	Conc. Fix & Perm Buffer B	2001301
○	Enhancer	2000482	○	Enhancer	2000482
○	Enhancer	2000482	-	empty	-
●	Conc. Quench Buffer B	2001300	●	Conc. Quench Buffer B	2001300
●	Conc. Quench Buffer B	2001300	●	Conc. Quench Buffer B	2001300
●	Conc. Quench Buffer B	2001300	●	Conc. Quench Buffer B	2001300

GEM-X Flex Sample Preparation v2 Kit – Module 2					
PN-1000781					
Shipped on dry ice; Store at -20°C					
Cap Color	Reagent	PN	Cap Color	Reagent	PN
●	Additive C	2001332	●	Additive C	2001332
●	Additive C	2001332	●	Additive C	2001332
-	empty	-			

The sample preparation kit provides sufficient reagents to process 96 samples.

For Sample Fixation

Vendor	Item	Part Number
Thermo Fisher Scientific	Formaldehyde (37% by Weight/Molecular Biology), Fisher BioReagents	BP531-25
	Nuclease-free Water (not DEPC-Treated)	AM9937
Corning	Phosphate-Buffered Saline, 1X <i>without Calcium and Magnesium</i>	21-040-CV

For Sample Filtration

Vendor	Item	Part Number
Sysmex	Sterile Single-Pack CellTrics Filters*	04-004-2326
Miltenyi Biotec	Pre-Separation Filters (30 µm)*	130-041-407
pluriSelect	pluriStrainer Mini 40 µm (Cell Strainer)*	43-10040
*Alternative products. Choose either based on availability & preference.		

96-well Deep Well Plates

Vendor	Item	Part Number
Axygen	96-well Clear V-Bottom 500 µL Polypropylene Deep Well Plate, Sterile**	P-96-450V-C-S
Corning	96-well Expanded Volume Polypropylene Not Treated Microplate, Standard Height, V-Bottom, Sterile**	3344

**Alternative products. Choose either based on availability & preference.

- All the deep-well plates are not individually packaged and do not have lids. To prevent potential RNase/sample cross contamination in such case, promptly clean up the working bench. The microplate lids can also be purchased separately.
- Alternative standard well plates may be used but the total reaction volumes may need to be modified. See Tips & Best Practices for more information.
- For overnight hybridization in a thermal cycler, samples can also be transferred to 96-well PCR plates, for example Eppendorf twin.tec PCR Plates 96 EP0030129504

Plate Seals & Applicators

Vendor	Item	Part Number
Bio-Rad	Microseal 'C' PCR Plate Sealing Film, adhesive***	MSC1001
	Microseal 'B' PCR Plate Sealing Film, adhesive***	MSB1001
	Film Sealing Roller for PCR Plates	MSR0001
Applied Biosystems	MicroAmp Optical Adhesive Film***	4311971
	MicroAmp Adhesive Film Applicator	4333183

***Alternative products. Choose either based on availability & preference.

Plate Lids

Vendor	Item	Part Number
Azenta Life Sciences	Microplate 96 Lid*	4ti-0290
Stellar Scientific	Clear Universal Fit Polystyrene Assay Plate Lid For 96 Well Assay Plates, RNase and DNase Free*	ILP-LID-UNI-10-N
Cytiva	Clear Polystyrene Universal Lid*	7704-1001

*Alternative products. Choose either based on availability & preference. Any equivalent sterilized universal 96-well plate lid can also be used.

Temperature-controlled Instruments for Incubation

Vendor	Item	Part Number
Eppendorf	Thermo Mixer w/ heated lid	5308000003
	ThermoMixer C Accessory, Smartblock Thermoblock	5363000039
	ThermoMixer C	5382000023
Analytik Jena	Biometra TAdvanced 96 SG/S	846-x-070-241/ 846-x-070-251 (x = 2 for 230 V; 4 for 115 V; 5 for 100 V, 50-60 Hz)
Eppendorf	Mastercycler X50s/X50a	6311000010/ 6313000018
Bio-Rad	PTC Tempo Deepwell Thermal Cycler	12015392
	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module (discontinued)	1851197
-	Oven (use any oven with consistent heating)	-
Choose either thermomixer, thermal cycler, or oven based on availability & preference. - If using thermal cyclers, choose Analytik Jena, Bio-Rad, or Eppendorf based on availability & preference. - For select instruments, ramp rates should be adjusted for all steps as described below: Analytik Jena Biometra TAdvanced 96 SG/S: 2°C/sec for both heating and cooling, Eppendorf Mastercycler X50s/X50a: 3°C/sec heating and 2°C/sec cooling		

Additional Materials

This list may not include some standard laboratory equipment.

Vendor	Item	Part Number
Eppendorf	DNA LoBind Tubes 2.0 ml	022431048
	DNA LoBind Tubes 1.5 ml	022431021
	DNA LoBind Tubes 5.0 ml	0030108310
Corning	Corning Centrifuge Tubes with CentriStar Cap (15 ml), sterile	430790
	Self-Standing Polypropylene Centrifuge Tubes (50 ml), sterile	430921
VWR	Vortex Mixer	10153-838

For Cell Counting

Vendor	Item	Part Number
Nexcelom Biosciences	ViaStain PI Staining Solution*	CS1-0109-5mL
	ViaStain AOPI 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* <i>Dilute 1:100 in PBS and use at 1:1 ratio with sample. Do not incubate before imaging/counting.</i>	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
	DAPI solution, 1 mg/mL*	62248
*Alternative stain products. Choose either based on availability & preference. If the sample has no debris, Trypan Blue can be used.		
**Choose Countess II/3, Cellaca, Cellometer or equivalent fluorescent counter.		

For Storage & Post-Storage Processing

Vendor	Item	Part Number
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%***	G5516-100ML
***Alternative products. Choose either based on availability & preference.		

Tips & Best Practices

Icons



Tips & Best Practices section includes additional guidance



Signifies critical step requiring accurate execution



Troubleshooting section includes additional guidance

Sample Quality

- Use high-quality single cell or nuclei suspensions that can withstand the fixation steps.
- Perform pilot experiments to determine if the sample type is suitable for the fixation.
- Highly viable single cell suspensions (>80%) will have the greatest sensitivity and cell recovery. However, the GEM-X Flex v2 assay is robust to samples at much lower viability, with successful results demonstrated even with low viability samples (50% or lower). Low viability samples may have more variable cell calling and lower sensitivity.
- Samples should have minimal debris for best results; debris can have associated RNA that can contribute to noncell background.

Plate-based Specific Guidance

Pipettes

- Use well-calibrated multichannel pipettes for the workflow.

96-well Plates

96-well Plate	Compatible with	Notes
Recommended deep-well ($\geq 350 \mu\text{l}$) plates		
Axygen/Corning V-bottom deep-well	Thermal Cycler, Thermomixer, and Oven	-
Alternative deep-well ($\geq 350 \mu\text{l}$) plates		
Flat- or round-bottom deep-well	Thermomixer and Oven*	• Minimal testing has been done with this plate type • No expected change in data quality • Cell loss might be greater than the V-bottom plates • Not recommended for low cell input (10,000-25,000)
Alternative standard-well ($\leq 350 \mu\text{l}$) plates		
V-bottom standard-well	Thermomixer and Oven*	• Minimal testing has been done with this plate type • No expected change in data quality • During fixation, scale down the Fixation Buffer B and Additive C volumes, keeping the ratio 1:2. To avoid overflow during centrifugation, ensure that the total volume added to each well is at least 50 μl less than the plate's maximum working volume. • For storage at -80°C, scale down the volume of reagents. See Appendix on page 19.
Flat- or round-bottom standard-well	Thermomixer and Oven*	• Minimal testing has been done with this plate type • No expected change in data quality • Cell loss might be greater than the V-bottom plates • During fixation, scale down the Fixation Buffer B and Additive C volumes, keeping the ratio 1:2. To avoid overflow during centrifugation, ensure that the total volume added to each well is at least 50 μl less than the plate's maximum working volume. • Not recommended for low cell input (10,000-25,000) • For storage at -80°C, scale down the volume of reagents. See Appendix on page 19.
<i>*If a thermal cycler is the only available option, transfer samples to a standard 96-well PCR plate ($\leq 350 \mu\text{l}$) or strip tubes before incubation.</i>		

- Heat the thermal cycler, oven, or thermomixer to appropriate temperature before using. The thermal cycler lid should also be set to the same temperature.
- When using a thermal cycler, ensure that the plate wells are in alignment with the thermal block.

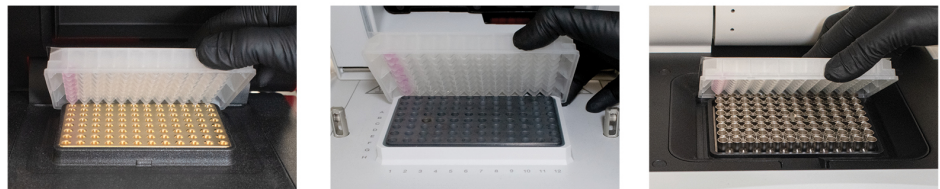
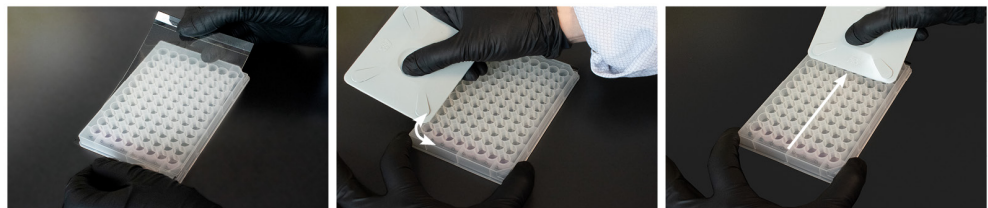
Placement of the Plate in the Thermal Cycler

Plate Sealing & Removal

- Use the recommended seals for sealing the plate.
- To apply the seal, place the plate flat on a clean work surface. Peel the backing from the adhesive seal. Align the seal with the plate and apply while firmly holding the plate with one hand. Press on the seal around the edge of each well to ensure uniform adhesion. Use a roller or an applicator to firmly press the seal along rows and columns so each well is sealed.
- To remove the seal, place the plate flat on a clean work surface. Hold the plate down firmly with one hand. Carefully pull the seal using the side tabs while continually holding the plate. Move slowly to ensure that no liquid splashes out of the well.

Sealing the Plate**Removing the Seal**

Practice Sample Handling in 96-well Plates

- If needed, practice sample handling in 96-well plates by using mock PBMC samples.
- Follow Fixation protocol steps using 4% formaldehyde for fixation and 1X PBS for wash steps instead of 10x Genomics reagents.

Fixation Conditions

- Fixation temperature and time depend upon the subsequent use of the fixed sample.

Fixed Sample Use	Fixation Time & Temperature
Fixed sample to be processed immediately	1 h at room temperature (20°C)
Fixed sample to be stored subsequently	16-24 h at 4°C



- DO NOT mix samples with different fixation times in one experiment.
- Guidance for optimal data quality:
 - If fixing antibody (intracellular or cell surface protein) labeled cells, fix for 16-24 h at 4°C.
 - If planning to store the fixed samples, fix for 16-24 h at 4°C.
 - Store fixed samples at -80°C.

Cell/Nuclei Number for Fixation

- Counting fresh single cell/nuclei suspensions prior to fixation can be skipped if viability information is not needed and if the amount of cells/nuclei does not overly exceed the upper limit recommendations going into fixation.

Fixed Cell Counting

- It is recommended that the sample be stained with a fluorescent dye such as AO/PI Staining Solution and counted using an automated fluorescent cell counter or hemocytometer.
- See [Cell Counting on page 19](#) for details.

Range

- For sample fixation, the recommended minimum number is 25,000 cells or nuclei to ensure there are enough cells/nuclei for the downstream workflow.
- The recommended maximum number for fixation is 10×10^6 cells per 0.5 ml Fixation Buffer B.
- It may be possible to use less than the recommended minimum numbers. The lower cell numbers during fixation will impact the cell input number during hybridization and increase the likelihood of cell/cell pellet loss prior to workflow completion.

Cell Loss

- Some cell loss is expected during the fixation steps depending up on the sample type, cell type, and user experience. When starting with 25,000 inputs into fixation, <25,000 fixed cell/nuclei will be moved forward into hybridization. See Sample Input section of the User Guides for more details when using <25,000 fixed cell/nuclei into hybridization.

Recommended Input

Recommended Cell/Nuclei Input

For Fixation

25,000–10 x 10⁶ cells

Per Hybridization

25,000–500,000 (If sufficient cells/nuclei are available, it is recommended to default to 300,000 fixed cells/nuclei)

≤100,000 (as higher cell loads may lead to a slight decrease in data quality), if using:

- Antibody-oligonucleotide labeled cells
- Splenocytes, human BMMCs, and leukocytes

Important Considerations

It may be possible to use ≤25,000 cells*, but it may lead to:

- Loss of pellet or a pellet that is not visible
- 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/Sample 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

**Limited in-house testing showed successful results from as few as 10,000 cells/nuclei*

Nuclei have been identified as a challenging sample type and can have a higher chance of clumping and post-fixation sample loss.

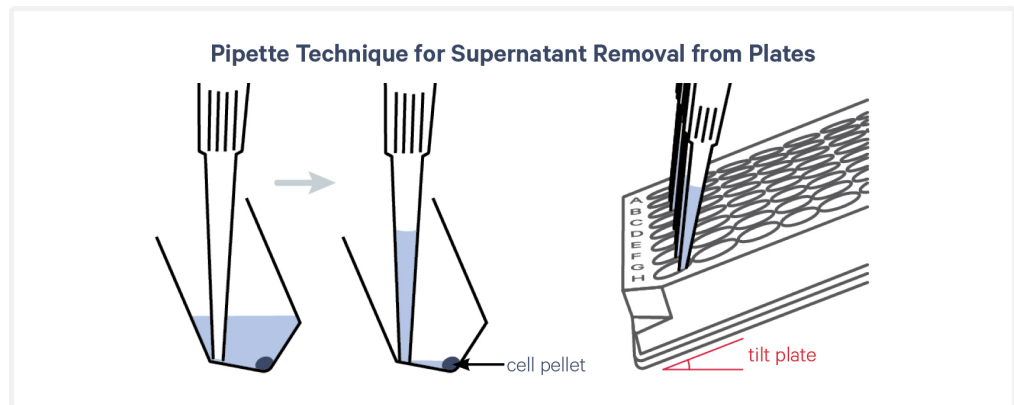
Mitigation Strategies when using Lower Cell Input

- Follow better sample preparation practices including use of a swinging bucket rotor
- During hybridization, up to 15 µl supernatant can be left behind to avoid losing the pellet
- During post-hybridization washes, up to 30 µl supernatant can be left behind to avoid losing the pellet
- Follow pooled wash workflow during post-hybridization wash

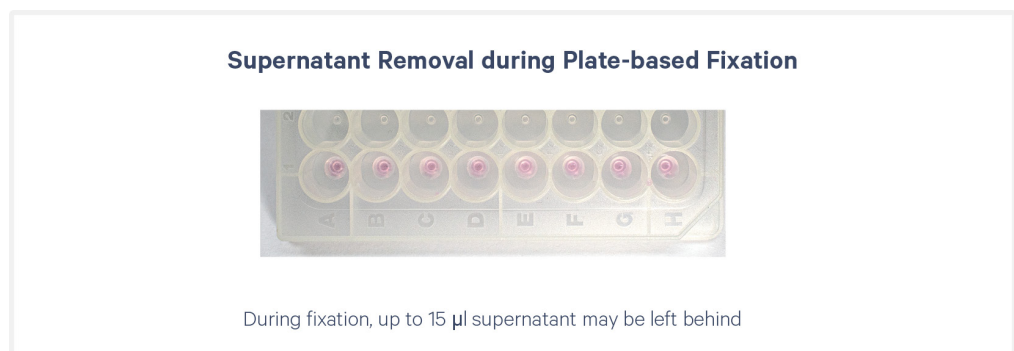
Centrifugation

- Use a swinging-bucket rotor for higher cell/nuclei recovery.
- Centrifugation speed and time may need optimization depending upon sample type. Larger or fragile cell types may require slower centrifugation speeds.
- Optimize sample-specific centrifugation conditions before moving to plates. The conditions optimized in tubes should apply to plates as well.

- Tilt the plate when removing the supernatant. Rest pipette tips on the bottom seam of the well to aspirate supernatant (see below) without disturbing the pellet.



- During fixation, up to 15 μ l supernatant may be left behind to optimize cell recovery without significantly impacting assay performance.



- If uncertain about the volume remaining in the wells/tubes, add 15 μ l water/PBS to an empty well/tube and use it for a visual estimation.
- After each buffer addition step, gently mix cells/nuclei 5x, or until the pellet is completely resuspended, without introducing bubbles.

Buffer Preparation

Prepare Buffers

All buffer preparations should be fresh.

Buffers for Fixation

Prepare Fixation Buffer

Fixation Buffer B						
Maintain at room temperature	Stock	Final	1X* + 10% (μl)	16X* + 10% (μl)	96X* + 10% (μl)	384X* + 10% (μl)
Nuclease-free Water	-	-	87.1	1,393.6	8,361.6	33,446.4
Conc. Fix & Perm Buffer B (10x Genomics PN-2001301)	10X	1X	11.0	176.0	1,056.0	4,224.0
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.						
Formaldehyde	37%	4%	11.9	190.4	1,142.4	4,569.6
Use formaldehyde with adequate ventilation, preferably in a fume hood. Follow appropriate regulations.						
Total	-	-	110.0	1,760.0	10,560.0	42,240.0
*X represents the number of sample wells						

Prepare Additive C

Additional Buffers	
Additive C (10x Genomics PN-2001332)	

- Thaw at **room temperature**.
- Vortex and check for precipitation.
- Maintain at room temperature. If precipitate is observed, heat at **42°C** for **10 min**.

Prepare Quenching Buffer

Quenching Buffer B**						
Maintain at 4°C	Stock	Final	1X* + 10% (µl)	16X* + 10% (µl)	96X* + 10% (µl)	384X* + 10% (µl)
Nuclease-free Water	-	-	192.5	3,080	18,480	73,920
Conc. Quench Buffer B (10x Genomics PN-2001300)	8X	1X	27.5	440	2,640	10,560
Thaw at room temperature. Vortex and centrifuge briefly. Maintain at 4°C.						
Total	-	-	220	3,520	21,120	84,480

*X represents the number of sample wells

**If planning to store the sample, 0.2 ml Quenching Buffer B will be required per sample during Post-Storage Processing.

Buffers for Storage of Fixed Samples

Prepare 50% Glycerol Solution

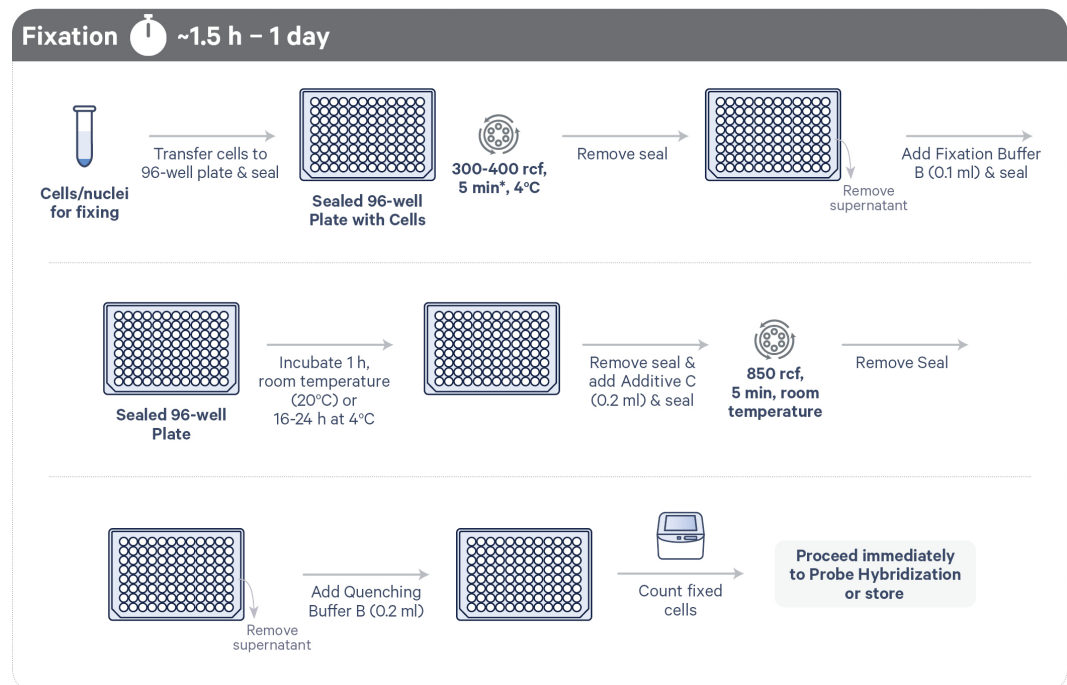
For long-term storage of fixed samples

- Mix an equal volume of nuclease-free water and 99% Glycerol, Molecular Biology Grade.
- Filter through a 0.2 µm filter.
- Store at room temperature in 2-ml DNA LoBind tubes.

Fixation in 96-well Plate

Introduction

GEM-X Flex Sample Preparation v2 Kit (PN-1000781) is used for sample fixation. This protocol is compatible with fresh cell and nuclei suspensions. The steps described here provide guidance for sample fixation and quenching in a 96-well plate.



Protocol: Fixation

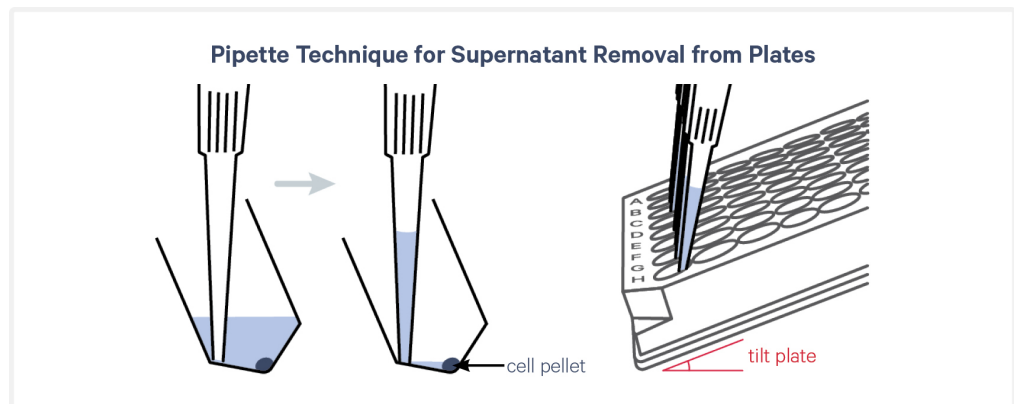
- a. Transfer 25,000– 2×10^6 cells into each well of a 96-well plate.
- b. Seal the plate with a plate seal.
- c. Centrifuge sample at **300–400 rcf** for **5 min** (PBMCs/cell lines) at **4°C**.
- d. Carefully remove the seal while steadily holding the plate.
- e. Remove the supernatant without disturbing the pellet.

TIPS

Tilt the plate when removing the supernatant. Rest pipette tips on the bottom seam of the well wall to aspirate supernatant as shown below without disturbing the pellet. Up to 15 μ l supernatant can be left behind.



Proceed immediately to the next step to prevent sample loss due to drying.



- f. Add **0.1 ml room temperature** Fixation Buffer B to the sample pellet and pipette mix 5x.
- g. Seal the plate with a plate seal.
- h. Incubate for **1 h** at **room temperature (20°C)** or for **16–24 h** at **4°C**. If planning to store fixed samples or fixing antibody labeled cells, a 16–24 h fixation at 4°C is recommended for optimal results.



DO NOT agitate or mix the sample during incubation.

If working with splenocytes, fix for 16–24 h at 4°C. If working with leukocytes or human BMMCs, fix for 16–24 h at room temperature (20°C).

To minimize variability for room temperature fixations, incubation at controlled 20°C temperature (e.g. with a thermomixer with a plate adaptor and no shaking or oven or thermal cycler) is recommended. Fixation time and temperature should be consistent across all samples in an experiment.

- i. Carefully remove the seal while steadily holding the plate.
- j. Add **0.2 ml room temperature** Additive C to the sample in Fixation Buffer B and pipette mix 5x.

At this stage, if necessary, samples may be maintained at room temperature for up to an hour. This short stopping point can be used to facilitate the efficient processing of a large number of samples.

- k. Seal the plate with a plate seal.
- l. Centrifuge at **850 rcf** for **5 min** at **room temperature**.
- m. Carefully remove the seal while steadily holding the plate.
- n. Remove the supernatant without disturbing the pellet.



Tilt the plate when removing the supernatant. Rest pipette tips on the bottom seam of the well wall to aspirate supernatant without disturbing the pellet. Up to 15 µl supernatant may be left behind.



Proceed immediately to the next step to prevent sample loss due to drying.

- o. Add **0.2 ml** chilled Quenching Buffer B to the sample pellet and pipette mix 5x and keep on ice.
- p. Determine cell concentration of the fixed sample using an automated cell counter or hemocytometer. See [Appendix on page 19](#) for Fixed Cell/Nuclei Counting.



For accurate cell counting, it is strongly recommended that the cell/nuclei suspension be stained with a fluorescent dye such as AO/PI Staining Solution and counted using an automated fluorescent cell counter.

- q. Proceed **immediately** to the appropriate User Guide and follow step 1 (Probe Hybridization) or store the sample after resuspending in appropriate reagents.



Samples can be stored at 4°C for up to 1 week or at -80°C for up to 12 months, depending upon the reagents used for storage. See [Appendix on page 19](#) for guidance on storage and post-storage processing.

Appendix

Cell Counting

- Accurate counting is critical for optimal assay performance.
- Combination of counters and dyes tested for counting fixed cells/nuclei, post-hybridization and post-hybridization wash.

Counter Type	Fluorescent Dye	Counting Comparison
Cellaca Range: 1×10^5 – 1×10^7 cells/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: 1×10^4 – 1×10^7 cells/ml (optimal 1×10^5 – 4×10^6) Manual debris exclusion from cell count postimage 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: 1×10^5 – 1×10^7 cells/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 are relatively dimmer and require longer exposure compared to NucSpot 470, so NucSpot 470 is preferred.

**Dilute the stock to 1:100 and mix 1:1 with the sample. For example, add 10 μ l diluted dye to 10 μ l sample.*

- It is strongly recommended that the fixed sample be stained with a fluorescent dye such as PI staining solution and counted using an automated fluorescent cell counter or hemocytometer. Fluorescent staining enables accurate counting even in the presence of sub-cellular debris.
- Focus cells under the brightfield before switching to the fluorescent channel.
- Increase exposure time to help adjust signal to noise during counting.

- Do a final visual inspection to confirm the counts are accurate. After obtaining the counts, switch between brightfield and fluorescent channel to ensure that the counts include minimal to no debris.
- Including debris in the count will result in lower chip loading numbers, which may contribute to lower cell recovery.
- Ensure that the cell counter emission/excitation filter is compatible with the fluorescent dye used.
- If using an automated cell counter, ensure that the cells are being circled correctly. The settings of the automated cell counters may need to be adjusted for optimal cell-detection accuracy.

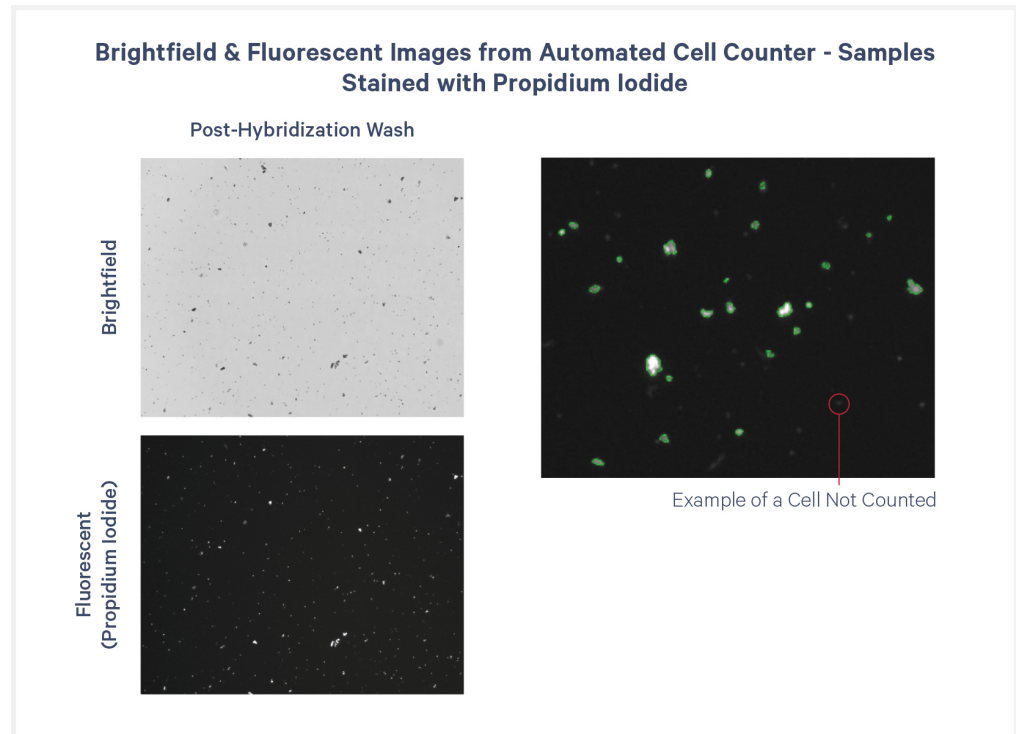
Some sample or cell resuspension buffers may have background autofluorescence that impacts counting accuracy; changing the fluorescence threshold/gating might not fix the background issue. In such cases, diluting the sample leads to stable counts over a broader range of fluorescence thresholds.

Counting Using PI Staining Solution

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

- Add **25 μ l** 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 μ l** sample to **15 μ l** PBS.
- Add **25 μ l** 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. See manufacturer's instructions for details.

Samples stained with PI Staining Solution can also be counted using Countess II FL, Countess 3 FL, and Cellometer K2 cell counters. See manufacturer's instructions for details.

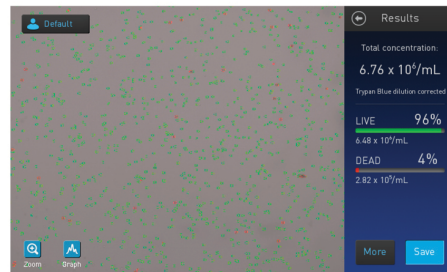
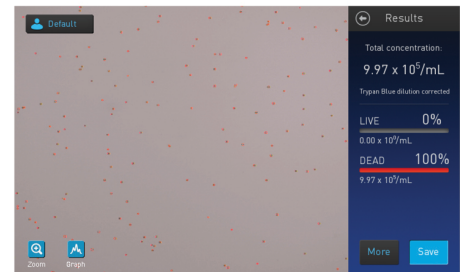


Counting Using Trypan Blue (Only for Debris-Free Samples)

Debris-free samples, for example, cell lines, can also be counted using trypan blue. Samples that are more like to have debris, for example, fixed & dissociated tissue samples, should be counted using fluorescent dyes.

This protocol below provides instructions for counting samples using trypan blue and a hemocytometer or Countess II Automated Cell Counter.

- Mix 1 part 0.4% trypan blue and 1 part sample.
- Transfer **10 μ l** sample to a Countess II Cell Counting Slide chamber or a hemocytometer.
- Insert the slide into the Countess II Cell Counter and determine the cell concentration. If using hemocytometer, count fixed cells by placing hemocytometer under the microscope.
- The majority of fixed cells will be stained with trypan blue stain and appear nonviable.

Brightfield image from Automated Cell Counter – Samples Stained with Trypan Blue**Fresh PBMCs****Fixed PBMCs**

Storage Guidance

Fixed samples can be stored for short or long-term. The volumes recommended below are for 96-well V-bottom deep-well (~350 µl) plates. If using standard-well plates (≤350 µl), the volumes can be scaled down while keeping the same ratios (see below). To avoid overflow during centrifugation, ensure that the total volume added to each well is at least 50 µl less than the plate's maximum working volume.

Sample Storage

- Fixed cell or nuclei suspensions 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.

Short-term Storage in 96-well Plates at 4°C:

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

- Add **0.1 volume** pre-warmed Enhancer to fixed sample in Quenching Buffer B.

For example, add **20 µl** Enhancer to **200 µl** fixed sample in Quenching Buffer B. Pipette mix.

Example volumes if using standard-well plates with a 250 µl working volume: Add 15 µl Enhancer to 150 µl fixed sample in Quenching Buffer B.

- Firmly seal the plate with a **new** micro film seal with a film sealing adaptor or roller. After proper sealing with a film, the plate can also be covered with a PCR plate lid.
- Store sample at **4°C** for up to **1 week**.

Long-term Storage in 96-well Plates at -80°C

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

- Add **0.1 volume** pre-warmed Enhancer to fixed sample in Quenching Buffer B.

For example, add 20 µl Enhancer to 200 µl fixed sample in Quenching Buffer B. Pipette mix.

Example volumes if using standard-well plates with a 250 µl working volume: Add 15 µl Enhancer to 150 µl fixed sample in Quenching Buffer B.

- c. Add 50% glycerol for a final concentration of 10%. For example: add 55 μ l 50% glycerol to 220 μ l fixed sample in Quenching Buffer B and Enhancer. Pipette mix.
Example volumes if using standard-well plates with a 250 μ l working volume: Add 41.3 μ l 50% glycerol to 165 μ l of sample in Quenching Buffer B + Enhancer.
- d. **Firmly** seal the plate with a **new** micro film seal with a film sealing adaptor or roller. After proper sealing with a film, the plate can also be covered with a PCR plate lid.
- e. Store at **-80°C** for up to **12 months**.



If planning to store the fixed samples, it is recommended to perform a 16-24 h fixation at 4°C during the fixation step and store the fixed samples at -80°C for best results.

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.

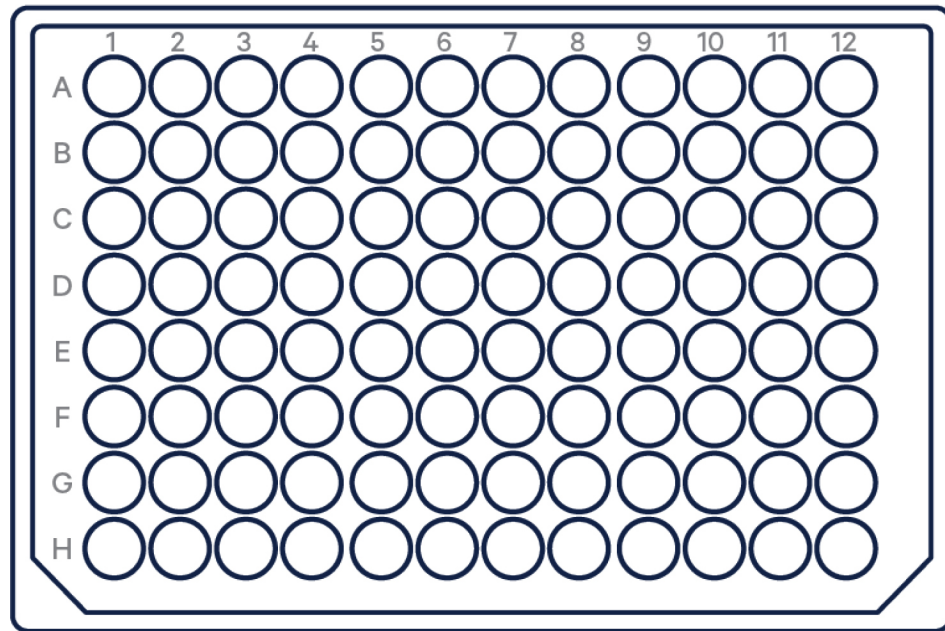
- a. Centrifuge sample at **850 rcf** for **5 min** at **room temperature**. Carefully remove the seal while steadily holding the plate.
- b. Remove the supernatant without disturbing the pellet.
Up to 30 μ l supernatant may be left behind if working with <300,000 cells.
- c. Resuspend cell pellet in **0.2 ml** Quenching Buffer B and keep on ice.
- d. Determine cell concentration of the fixed sample using an automated cell counter or hemocytometer. See [Cell Counting on page 19](#).
- e. Proceed **immediately** to the appropriated GEM-X Flex v2 User Guide (see References).

Shipping Guidance

- Fixed samples resuspended in Quenching Buffer B supplemented with Enhancer can be shipped with a cold pack. See Short-term Storage for details.
- Fixed samples resuspended in Quenching Buffer B supplemented with Enhancer and Glycerol can be shipped on dry ice. See Long-term Storage for details.

Visual Layout of a 96-well V-bottom Deep-well Plate

This plate layout may be used to mark the position of samples and probes.



References

- a. GEM-X Flex v2 - Protocol Planner (CG000832)
- b. Cell Surface & Intracellular Protein Labeling for GEM-X Flex Gene Expression (CG000781)
- c. GEM-X Flex v2 User Guide (CG000834)
- d. GEM-X Flex v2 with Feature Barcode technology for Protein (CG000835)

Document Revision Summary

Document Number	CG000833
Title	Plate-based Sample Fixation for GEM-X Flex v2
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Revision Date	October 2025
Description of Changes	n/a

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