Extended Collection-to-Processing Windows with Blood Collection Tubes for 10x Single Cell Assays

Introduction

Peripheral blood is a critical sample type for research and clinical applications, but conventional EDTA tubes have a narrow processing window (within ~2 hours). This Technical Note evaluates two blood collection tubes that extend this window: Streck Cell-Free DNA (Streck), which supports peripheral blood mononuclear cell (PBMC) workflows, and PAXgene Blood RNA (PAXgene), which supports leukocyte workflows and adds flexibility with fresh or frozen blood. Together, they enable flexible logistics for distributed, multi-site studies.

10x Genomics Single Cell assays enable high-resolution profiling of gene expression, immune repertoire, and protein expression across a range of sample types, including blood. Peripheral blood is important for disease monitoring, biomarker discovery, and clinical research. Conventional EDTA blood collection has a narrow storage window, the period between collection and processing, beyond which sample integrity rapidly deteriorates and stress-response gene expression is artificially elevated. This is a challenge for multi-site studies, where collection and processing occur in different locations. Blood collection tubes (BCTs) that stabilize samples immediately after collection can ease this constraint by preserving the biological state of blood during transport and permitting delayed downstream processing.



Figure 1. Compatibility of BCTs with 10x Genomics GEM-X Flex v2 and GEM-X 5' v3 assays. Overview of the BCTs evaluated, storage windows prior to processing, and compatibility with 10x Genomics single cell assays.

Streck BCT (Streck, 230469) and PAXgene Tube (PreAnalytiX, 762165) are specialized blood collection tubes designed to stabilize blood for downstream testing and analysis. Streck reduces the release of contaminating cellular genomic DNA during storage. PAXgene stabilizes intracellular RNA immediately upon collection and samples can be frozen for long-term storage (–20°C to –80°C), making it suitable for archival sample analysis. However, the stabilization process makes PAXgene incompatible with cell type enrichment and cell surface protein labeling, so only leukocytes can be isolated.

In this Technical Note, Streck and PAXgene BCTs were evaluated against the conventional BD Vacutainer K2 EDTA BCT (EDTA; BD, 366643) for single cell profiling of blood samples using GEM-X Flex v2 (Apex) and GEM-X 5' v3 assays (Figure 1).

Data presented show Streck extends the storage window for PBMCs (subset of leukocytes excluding granulocytes) to 2 days at 4°C for GEM-X Flex v2 Gene Expression or Gene and Protein Expression.

PAXgene extends the window for leukocytes (all white blood cells, including granulocytes) to 3 days at 4°C or 20°C before needing to freeze for long-term storage or processing for GEM-X Flex v2 Gene Expression and GEM-X 5' v3 Gene Expression and V(D)J profiling. Additionally, PAXgene blood can be frozen upon collection for banking and delayed processing for months and potentially years, unlike Streck, which requires blood fixation within 2 days of collection before long-term storage.

Data and methods presented in this document allow researchers to select the appropriate BCT based on their experimental needs.

Methods Overview

Human whole blood was collected into Streck, PAXgene, or EDTA tubes and held for the storage window each tube supports before being subjected to a sample processing workflow. Blood from the same donor was used for all conditions within each experiment.

Depending on the tube type, cell type, and downstream assay, samples were processed with one of three workflows (Workflows I–III). Workflow specifics, including reference documents, are provided in the Appendix.

For Streck and EDTA tubes, applicable blood fixation, isolation, and protein labeling protocols were followed.

For PAXgene, blood was stabilized directly in the tube and optionally stored at 4°C or 20°C for up to 3 days. Blood was frozen at –20°C or, for long-term storage, at –80°C after freezing overnight at –20°C. Frozen blood was thawed up to 3 times in the freeze-thaw experiments, then processed directly for leukocyte isolation as specified in the step-by-step guidance in the Appendix.

Streck and EDTA blood require a multi-step bead-based isolation workflow (consult CG000785 and CG000392), while PAXgene leukocyte isolation needs a single centrifugation-based wash for GEM-X Flex v2 and does not require formaldehyde fixation (see Appendix), making PAXgene better suited for high-throughput studies.

Data were analyzed using Cell Ranger 10.1. All cell type annotations were performed using Cellarium Cell Annotation Service (CAS) for reference-based annotation. Differentially expressed genes (DEGs) were identified using the Wilcoxon rank-sum test and defined as genes with $|\log_2 FC| > 1$ and $p < 0.05$.

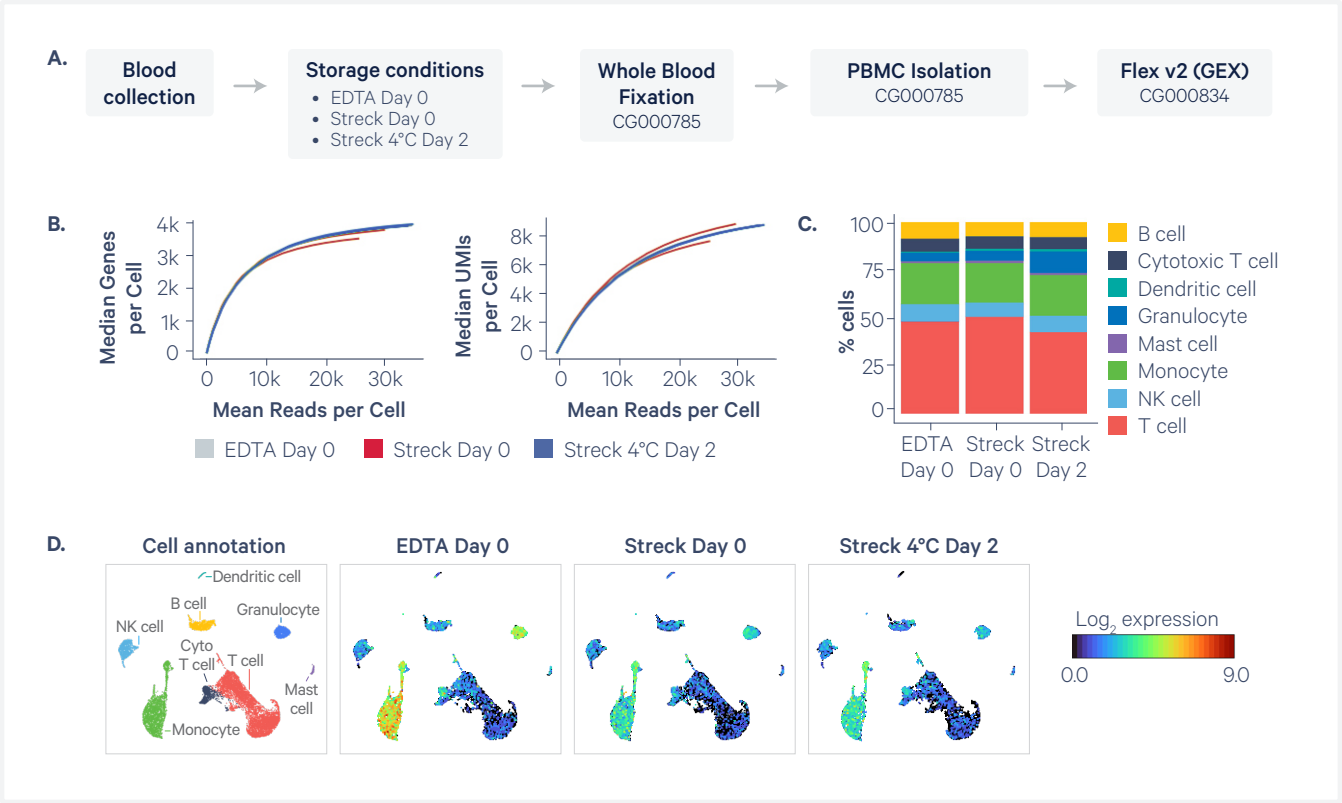


Figure 2. Streck PBMC performance with GEM-X Flex v2 Gene Expression after 2-day storage at 4°C. A. PBMCs isolated from EDTA and Streck fixed blood were profiled with GEM-X Flex v2 Gene Expression. **B.** Median genes and UMI (unique molecular identifier) counts per cell. **C.** Immune cell type proportions. **D.** UMAP projections of cell annotation and $\log_2$ sum expression of stress genes *(DUSP1, JUN, FOS, FOSB, EGR1,* and *CXCL8).*

Results

Streck BCTs Deliver High-Quality PBMC Data for GEM-X Flex v2

Streck was originally designed for cell-free DNA collection for liquid biopsy. To evaluate its suitability for single cell profiling and the impact of sample storage, matched blood was collected and processed in the following tubes/conditions: EDTA processed immediately (EDTA Day 0), Streck processed immediately (Streck Day 0), and Streck stored at 4°C for 2 days before processing (Streck Day 2). Following storage, blood was fixed prior to bead-based PBMC isolation (Workflow I), then analyzed using the GEM-X Flex v2 Gene Expression assay (Figure 2A).

Median genes and unique molecular identifiers (UMIs) detected per cell track closely among EDTA Day 0, Streck Day 0, and Streck 4°C Day 2 (Figure 2B), and immune cell type proportions are also preserved across all three conditions (Figure 2C). Gene expression changes minimally after 2-day storage at 4°C (< 130 DEGs). These data indicate that 2-day 4°C storage of Streck blood produces high-quality Flex v2 Gene Expression data without compromising sensitivity or sample composition.

Monocytes, a major PBMC population, are particularly sensitive to mechanical/ex vivo stress during blood handling. Stress gene expression (*DUSP1, JUN, FOS, FOSB, EGR1*, and *CXCL8*) is substantially reduced in monocytes in both Streck

Day 0 and 4°C Day 2 compared with EDTA Day 0 (Figure 2D), indicating Streck BCTs effectively suppress collection-induced stress gene expression in PBMC samples compared to EDTA, even after 2 days at 4°C. Blood in EDTA tubes shows elevated stress gene expression even at Day 0 (processed < 2 hours after blood collection), which is exacerbated by standard overnight shipment at 4°C (data not shown), reducing sample integrity relative to Streck blood.

Streck was also assessed for compatibility with cell surface and intracellular protein labeling using the MultiPro Human Discovery Panel (10x Genomics PN-1001205/PN-1001206). PBMCs were isolated, fixed, and stained with the Human Discovery Panel containing 347 DNA-barcoded antibodies for the GEM-X Flex v2 workflow (Figure 3A; Workflow II). It may be possible to stain blood that has been fixed prior to PBMC isolation, but panel performance may be compromised; this was not characterized here.

Figure 3C highlights two representative B cell markers: CD19, an extracellular epitope, and POU2AF1, an intracellular epitope accessible to antibody labeling following fixation and permeabilization. For both markers, the

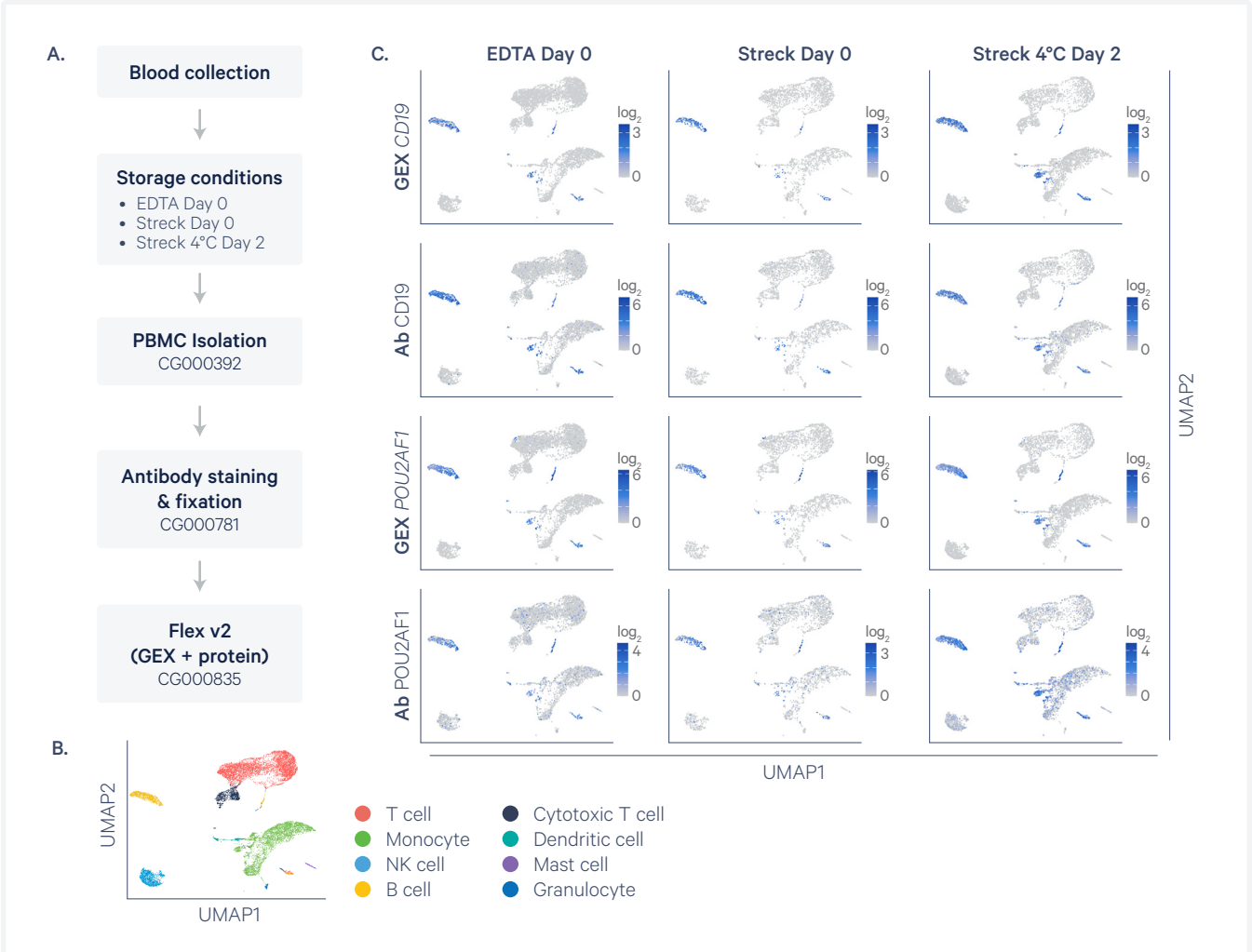


Figure 3. Streck PBMC performance with GEM-X Flex v2 Gene and Protein Expression after 2-day storage at 4°C. A. PBMCs isolated from EDTA and Streck blood were fixed and labeled with antibodies, and profiled with GEM-X Flex v2 Gene and Protein Expression. **B.** UMAP projection of cell annotation. **C.** Gene and antibody expression of extracellular marker CD19 and intracellular marker POU2AF1.

antibody signal closely matches gene expression and is enriched in the B cell cluster (Figures 3B and 3C) across all three conditions. Antibody signal-to-noise is comparable between Streck Day 0 and EDTA Day 0. Although a slight reduction in signal-to-noise was observed in Streck Day 2, similar proportions of cell types were correctly annotated from antibody data in EDTA Day 0, Streck Day 0, and Streck Day 2 (data not shown).

Together, these results show that Streck blood stored for 2 days at 4°C is compatible with the MultiPro Human Discovery Panel for simultaneous profiling of PBMCs for both gene and protein (intra- and extracellular) expression, using GEM-X Flex v2.

Streck blood held at 4°C is not recommended for leukocyte isolation. Cold temperature has been shown to reduce gene expression sensitivity in granulocytes. Streck is also not recommended for GEM-X 5' v3. Gene expression sensitivity is ~50% lower and V(D)J sensitivity ~30% lower than with PAXgene and EDTA. However, all major immune populations are still detected, with comparable cell type proportions and a > 70% V(D)J pairing rate. If Streck blood is being considered for the GEM-X 5' v3 assay, a pilot study is strongly recommended.

PAXgene BCTs Deliver High-Quality Leukocyte Data for GEM-X Flex v2 Gene Expression

PAXgene tubes, which are CE-marked for in vitro diagnostic use and are part of an FDA-cleared blood RNA collection and purification system, rapidly stabilize intracellular RNA upon collection, helping preserve gene expression profiles during storage and transport. Recent work has shown that single nucleus 3' gene expression data can be obtained from PAXgene blood (1).

To assess whether PAXgene enables GEM-X Flex v2 Gene Expression profiling of leukocytes with extended storage time, PAXgene blood was processed at Day 0 (PAXgene Day 0) or after 3 days at 4°C (PAXgene 4°C Day 3) or 20°C (PAXgene 20°C Day 3). Leukocytes were isolated using Workflow III, which requires a single centrifugation-based wash for GEM-X Flex v2 and no separate fixation step. Day 0 EDTA (EDTA Day 0; Workflow I) served as benchmarks (Figure 4A).

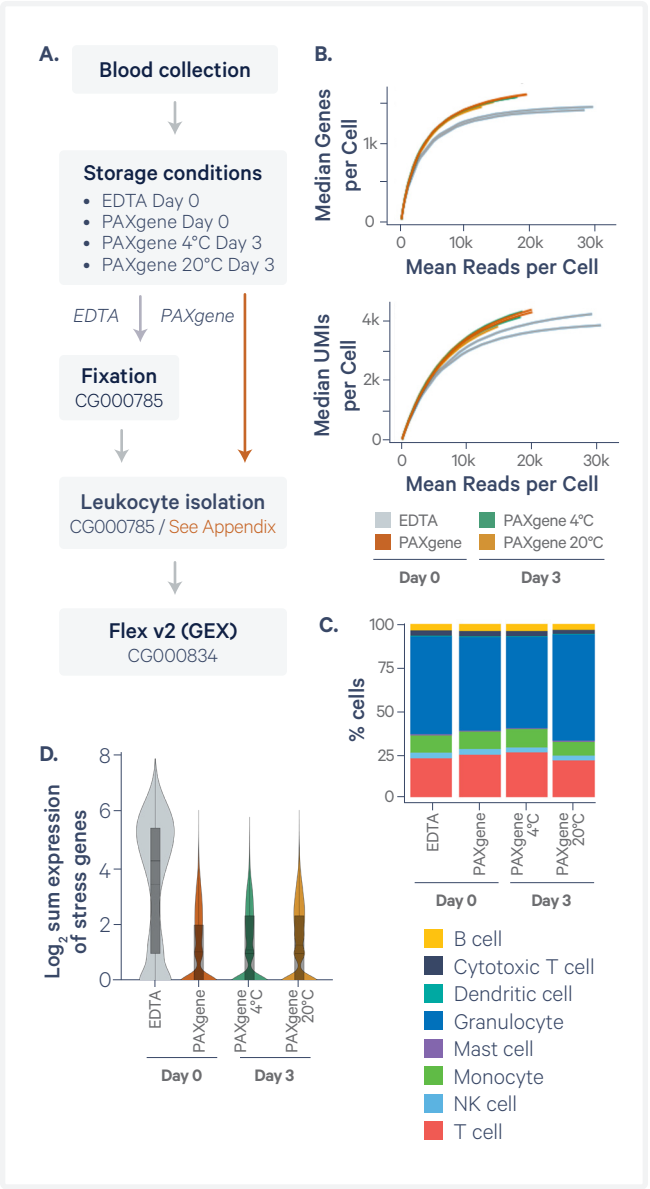


Figure 4. PAXgene leukocyte performance with GEM-X Flex v2 Gene Expression after 3-day storage. A. Blood collected in EDTA or PAXgene tubes was stored for 0 or 3 days at the indicated temperatures. EDTA blood was fixed, followed by bead-based leukocyte isolation. PAXgene leukocytes were isolated with centrifugation-based wash. **B.** Median genes and UMI counts per cell. **C.** Immune cell type proportions, after removing RBCs (red blood cells) post-sequencing. **D.** Log_2 sum expression of stress genes *(DUSP1, JUN, FOS, FOSB, EGR1,* and *CXCL8)* by storage condition.

PAXgene leukocytes yield higher overall median gene and UMI counts per cell than EDTA leukocytes (Figure 4B). Sensitivity after 3-day storage at either 4°C or 20°C is comparable to PAXgene Day 0 (Figure 4B), and immune cell type composition is consistent across all conditions (Figure 4C). Gene expression also changes minimally after 3 days at either temperature compared with Day 0, although 20°C produced more DEGs than 4°C (< 60 DEGs at 20°C vs. < 5 DEGs at 4°C). Together with the sensitivity (Figure 4B) and cell type composition (Figure 4C) results, this indicates that a 3-day hold at either temperature does not compromise data quality.

Stress-response gene expression is markedly higher in EDTA leukocytes than in PAXgene leukocytes at Day 0 and after 3 days of storage at either 4°C or 20°C, with the highest values in granulocytes and monocytes (Figure 4D). EDTA BCTs contain only an anticoagulant, so fragile cell types such as granulocytes and monocytes remain metabolically active and mount a stress response between draw and processing. In contrast, PAXgene stabilizes the transcriptome at the time of draw, preserving biology closer to its in vivo state.

In addition to the 3-day storage window, PAXgene blood can be frozen immediately after collection for long-term storage and subjected to up to 3 freeze-thaw cycles (Workflow III), reducing the need to aliquot or relabel for sample tracking. Leukocytes isolated after 1, 2, or 3 freeze-thaw cycles show comparable assay sensitivity (Figure 5A) and immune cell type composition (Figure 5B), with minimal changes in gene expression across cycles (< 70 DEGs detected). These data indicate that freeze-thaw handling has minimal effect on assay performance or gene expression.

Two considerations apply to PAXgene for GEM-X Flex v2. First, PAXgene blood is not compatible with cell type enrichment or cell surface protein labeling, so only leukocytes can be isolated. Second, PAXgene leukocyte preparations also contain more red blood cell (RBC) transcripts (*HBB*, *HBD*, *HBA2*, and *HBG1*) and platelet transcripts (*PPBP*) than EDTA. This contamination typically accounts for only ~10% of usable data and can be filtered during post-sequencing analysis, a minor limitation given PAXgene's higher sensitivity and greater logistical flexibility.

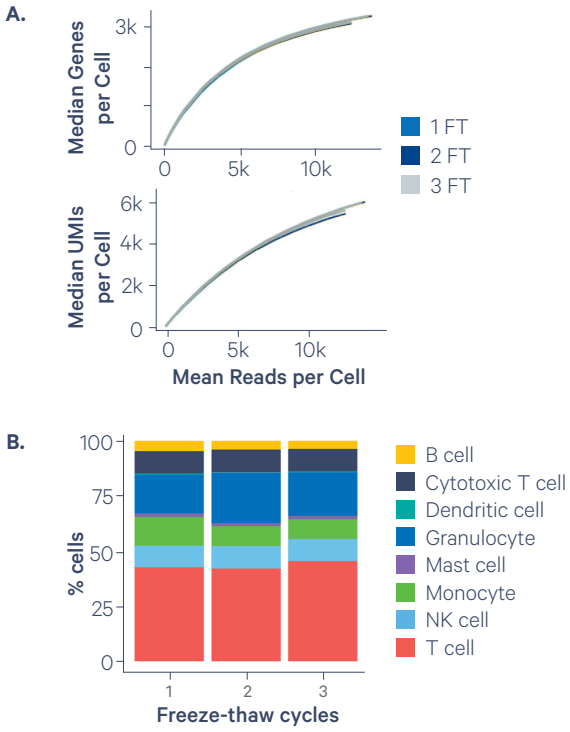


Figure 5. PAXgene leukocyte performance with GEM-X Flex v2 Gene Expression after repeated freeze-thaw (FT) cycles. PAXgene blood samples underwent up to three FT cycles before leukocyte isolation (Workflow III). **A.** Median genes and UMI counts per cell after 1, 2, or 3 FT cycles. **B.** Immune cell type proportions after 1, 2, or 3 FT cycles, after removing RBCs post-sequencing.

Overall, the data indicate that PAXgene provides logistical flexibility for GEM-X Flex v2 Gene Expression profiling of leukocytes. PAXgene blood can be stored for up to 3 days at 4°C or 20°C, or frozen immediately after collection for batch processing and thawed up to 3 times, without compromising sensitivity or cell type recovery. PAXgene also reduces the stress response seen with EDTA, preserving granulocyte expression profiles closer to the in vivo state. For optimal data quality,

store blood at 4°C when possible, including before freezing. When shipping, use cold packs to keep samples near 4°C. Keep storage temperature and freeze-thaw handling consistent within a study.

PAXgene BCTs Deliver High-Quality Leukocyte V(D)J Data for GEM-X 5' v3

PAXgene performance for V(D)J profiling was assessed using GEM-X 5' v3 in leukocytes isolated from PAXgene blood Day 0 (Workflow III), compared with EDTA blood Day 0 (Workflow II) (Figure 6A).

At comparable sequencing depth, PAXgene leukocytes show higher median genes and median UMIs per cell relative to EDTA leukocytes, which is driven mainly by the ~50% granulocyte population (data not shown). This is consistent with PAXgene stabilizing fragile granulocytes against the ex vivo RNA degradation seen in EDTA blood. PAXgene preparation also does not alter leukocyte composition. All major populations are recovered at proportions comparable to EDTA after RBC removal post-sequencing (Figure 6C), indicating that PAXgene leukocyte isolation does not introduce systematic bias in cell type recovery.

For GEM-X 5' v3, PAXgene leukocytes showed V(D)J performance comparable to EDTA controls, including similar rates of cells with a productive V-J spanning pair in both T cell receptor (TCR) and B cell receptor (BCR) libraries (Figure 6B). Median UMIs per cell were lower in PAXgene leukocytes for the immunoglobulin (IGH, IGK, IGL) and TCR (TRA, TRB) chains (Figure 6B). However, TCR clonotype frequencies remained comparable to EDTA leukocytes (Figure 6D), with no evidence of clonotype depletion. This donor shows low BCR clonotype expansion (data not shown), limiting the assessment of BCR clonotype changes. These results indicate that PAXgene preservation supports V(D)J profiling without compromising clonotype recovery.

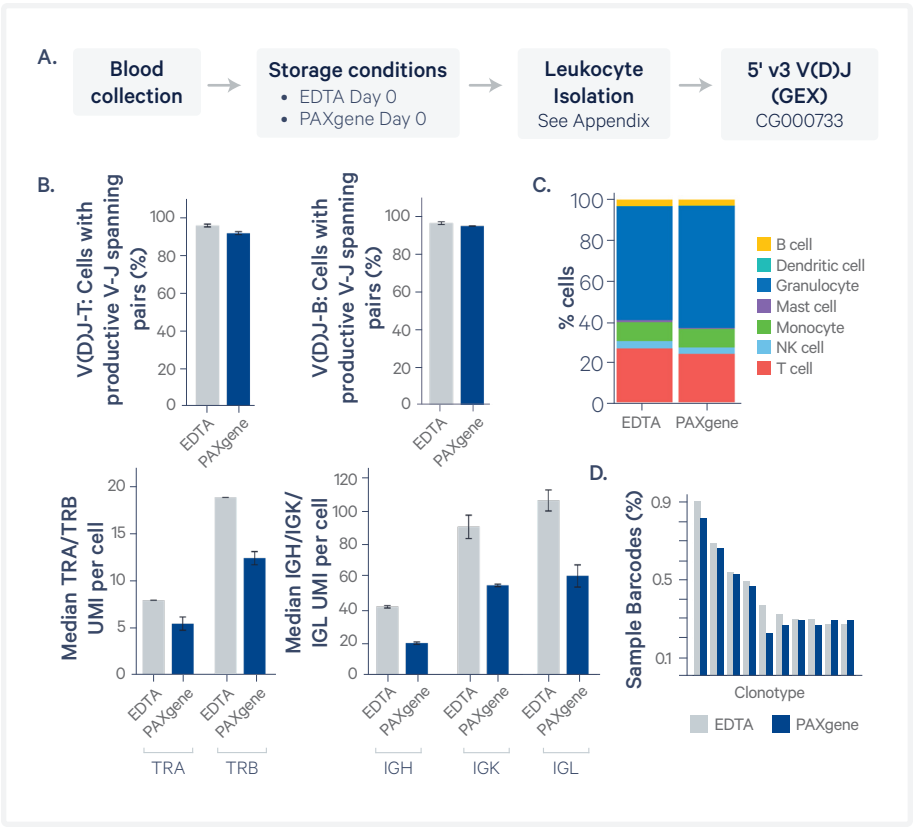


Figure 6. PAXgene leukocyte V(D)J profiling using the GEM-X 5' v3 assay at Day 0. A. Leukocytes were isolated from EDTA by bead-based RBC removal and from PAXgene blood by centrifugation-based wash for V(D)J profiling. **B.** Fraction of cells with a productive V-J spanning pair (V(D)J-B for BCR and V(D)J-T for TCR) and median UMIs per cell for BCR (IGH, IGK, IGL) and TCR (TRA, TRB) chains. **C.** Immune cell type proportions, after removing RBCs post-sequencing. **D.** TCR clonotype frequency as a percentage of sample barcodes.

To assess whether PAXgene blood provides similar storage and freeze-thaw flexibility with GEM-X 5' v3, blood was frozen immediately after collection (Freeze immediately) or stored for 3 days at 4°C or 20°C before freezing (3 days 4°C/20°C pre-freeze), with each condition subjected to up to 3 freeze-thaw cycles. Leukocytes were then isolated (Workflow III) and analyzed using the GEM-X 5' v3 Gene Expression assay (Figure 7A).

Compared with blood frozen immediately, PAXgene blood stored for up to 3 days at 4°C or 20°C before freezing maintains V(D)J library quality (Figure 7B). Both TCR and BCR metrics decline more at 20°C than at 4°C, including median UMIs per cell for TCR alpha (TRA) and beta (TRB) chains and BCR IGH, IGK, and IGL chains. TCR and BCR mapping metrics were comparable across all conditions (Figure 7B), as were TCR clonotype distributions (Figure 7C). This donor shows very low BCR clonotype expansion (data not shown), limiting the assessment of BCR clonotype diversity.

Storage at 4°C before freezing produced the smallest change in gene expression relative to blood frozen immediately (< 40 DEGs detected). Storage

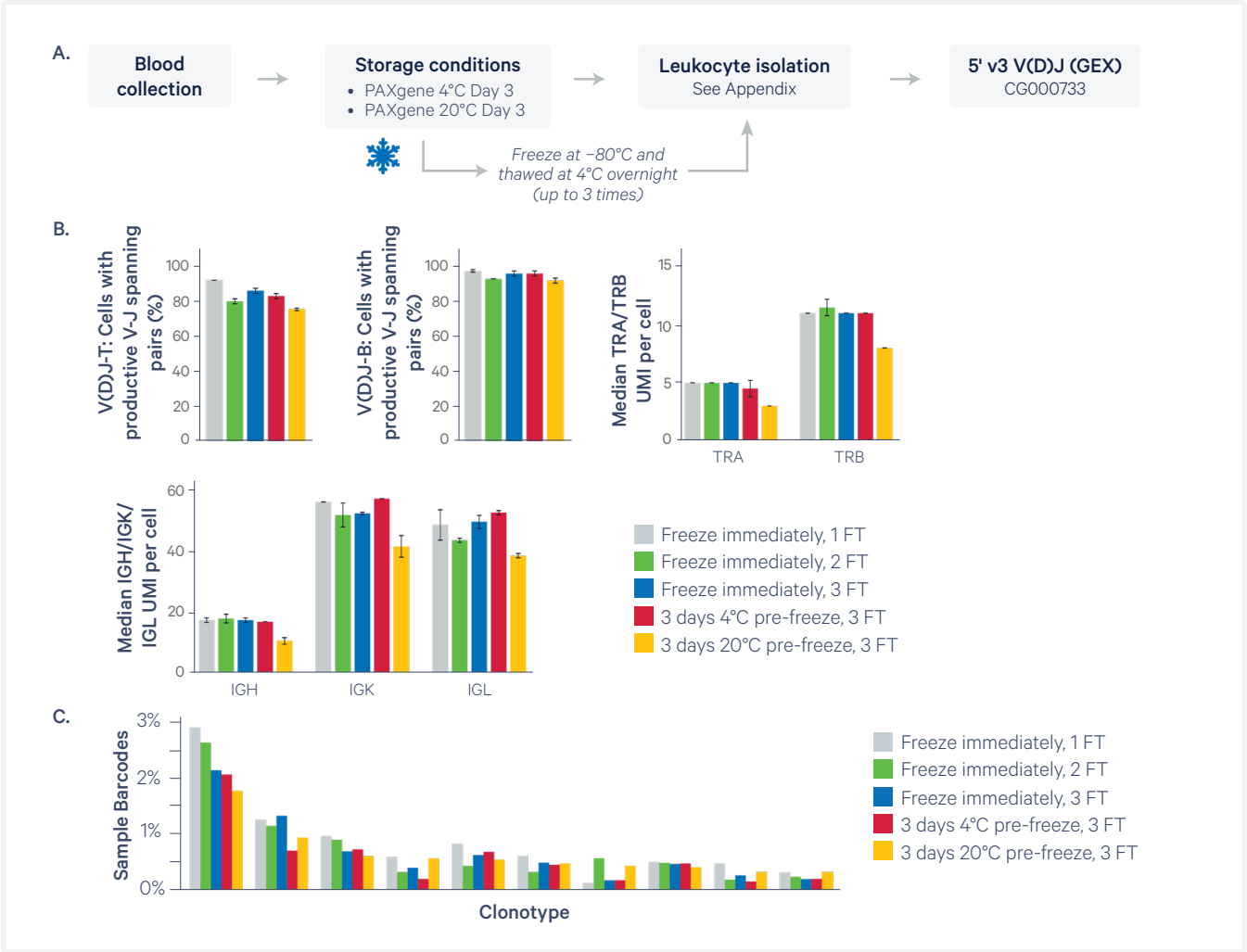


Figure 7. PAXgene leukocyte V(D)J profiling with GEM-X 5' v3 after 3-day storage and multiple freeze-thaw (FT) cycles. **A.** PAXgene blood was frozen immediately or stored for 3 days at 4°C or 20°C before freezing. Samples were thawed and leukocytes were isolated by centrifugation-based wash for V(D)J profiling. **B.** Fraction of cells with a productive V-J spanning pair (V(D)J-T for TCR and V(D)J-B for BCR) and median UMIs per cell for TCR (TRA, TRB) and BCR (IGH, IGK, IGL) chains after 3-day storage or 1, 2, or 3 FT cycles. **C.** TCR clonotype expansion and frequency as a percentage of sample barcodes.

at 20°C before freezing produced more DEGs (< 350 DEGs detected). Among these, RBC transcripts were higher in blood frozen immediately or stored at 4°C than in blood stored at 20°C, possibly reflecting more efficient RBC lysis during storage at 20°C. Immune cell type distribution was broadly comparable across storage temperatures (data not shown), indicating that biological interpretation is unaffected.

PAXgene blood also tolerates immediate freezing for long-term storage and up to 3 freeze-thaw cycles. After 1, 2, or 3 freeze-thaw cycles, leukocytes isolated by Workflow III show comparable TCR and BCR mapping metrics (productive V-J spanning pairs) and median UMIs per cell (Figure 7B). This indicates that freeze-thaw handling does not compromise V(D)J library parameters. Immune cell type distribution was broadly comparable across freeze-thaw conditions (data not shown), indicating that biological interpretation is unaffected.

Similar to GEM-X Flex v2, two considerations apply to PAXgene for GEM-X 5' v3. First, PAXgene blood is not compatible with cell surface protein labeling, so only leukocytes can be isolated. When antibody-based protein capture is needed alongside V(D)J, EDTA blood remains the better option, though it has tighter storage windows and shows more stress gene expression. Second, as observed with GEM-X Flex v2, immediate lysis in PAXgene tubes results in more RBC transcripts than in EDTA controls. These transcripts *(HBB, HBD, HBA2, and HBG1)* account for 10–15% of UMIs, a small and predictable loss of usable data. They can be filtered during analysis, which helps ensure accurate clustering and cell type annotation.

Overall, these data indicate that PAXgene supports V(D)J profiling after up to 3 days of storage at 4°C or 20°C and up to 3 freeze-thaw cycles, without compromising clonotype recovery. Storage at 4°C is recommended where possible for optimal data quality. To limit variability, keeping storage temperature and duration consistent across samples within a study is recommended.

Multi-Assay Compatibility of PAXgene Leukocytes Across GEM-X Flex v2, 3' v4, and 5' v3 Assays

To assess whether a single PAXgene blood sample can support multiple assays, leukocytes were isolated (Workflow III) and analyzed with GEM-X Flex v2, GEM-X 3' v4, and GEM-X 5' v3.

In PAXgene leukocytes, GEM-X Flex v2 yields the highest UMI and gene counts per cell across all major cell types, while the sensitivity of GEM-X 3' v4 and 5' v3 is comparable to each other and 1.5–2-fold lower (Figure 8A). Cell type composition is consistent across all three assays (Figure 8B), indicating strong biological concordance among the assays. Researchers can therefore choose the assay best suited to their study without changing the collection protocol.

EDTA leukocytes (Workflow II) were profiled with the same assays for reference. In EDTA leukocytes, granulocyte sensitivity is notably lower with GEM-X 3' v4 and 5' v3 than with Flex v2 (Figure 8C), but both assays still generate annotatable data with comparable cell type proportions (data not shown). PAXgene leukocytes also show lower stress gene expression than EDTA leukocytes (data not shown), making PAXgene well suited to granulocyte-focused studies, particularly with GEM-X Flex v2 for the highest granulocyte gene expression sensitivity.

Two considerations apply to PAXgene for GEM-X 3' v4. First, leukocytes isolated with two centrifugation washes (Workflow III) have ~30% of UMIs from RBC transcripts, compared with ~10% in GEM-X Flex v2 and ~15% in GEM-X 5' v3. Depleting globin RNA with a CRISPR-based method is one option to reduce this contamination (1). Second,

extended storage and freeze-thaw were not evaluated with the GEM-X 3' v4 assay, so a pilot experiment is recommended before a larger study.

Together, these results indicate that PAXgene blood supports all three single cell assays at Day 0, with storage and freeze-thaw validated for GEM-X Flex v2 and GEM-X 5' v3. GEM-X Flex v2 provides the highest sensitivity and multiplexing capacity, while GEM-X 3' v4 and 5' v3 recover comparable cell populations and support their assay-specific features, long-read compatibility and V(D)J profiling, respectively.

Conclusion

This Technical Note shows that both Streck and PAXgene extend the blood storage window beyond standard EDTA collection, with the best tube depending on cell type, assay, and study logistics.

For PBMCs analyzed with GEM-X Flex v2 Gene Expression or Gene and Protein Expression, Streck is the recommended BCT. After 2 days at 4°C, it maintains PBMC composition and transcriptomic sensitivity, supports antibody labeling of extracellular and intracellular epitopes, and limits the stress-response signature seen in non-stabilized EDTA blood. Storing Streck blood at 4°C best maintains gene expression sensitivity in PBMCs, particularly monocytes, but reduces sensitivity in granulocytes. For leukocyte gene expression studies, PAXgene tubes are therefore recommended for greater logistical flexibility and highest gene expression sensitivity in granulocytes.

For leukocytes analyzed with GEM-X Flex v2 Gene Expression or GEM-X 5' v3 Gene Expression and V(D)J, PAXgene is the recommended BCT. It supports storage for up to 3 days at 4°C or 20°C and up to 3 freeze-thaw cycles without compromising sensitivity or cell type recovery. PAXgene blood can also be frozen immediately after collection for long-term storage (months and potentially years at −80°C), so blood can be collected, frozen, and

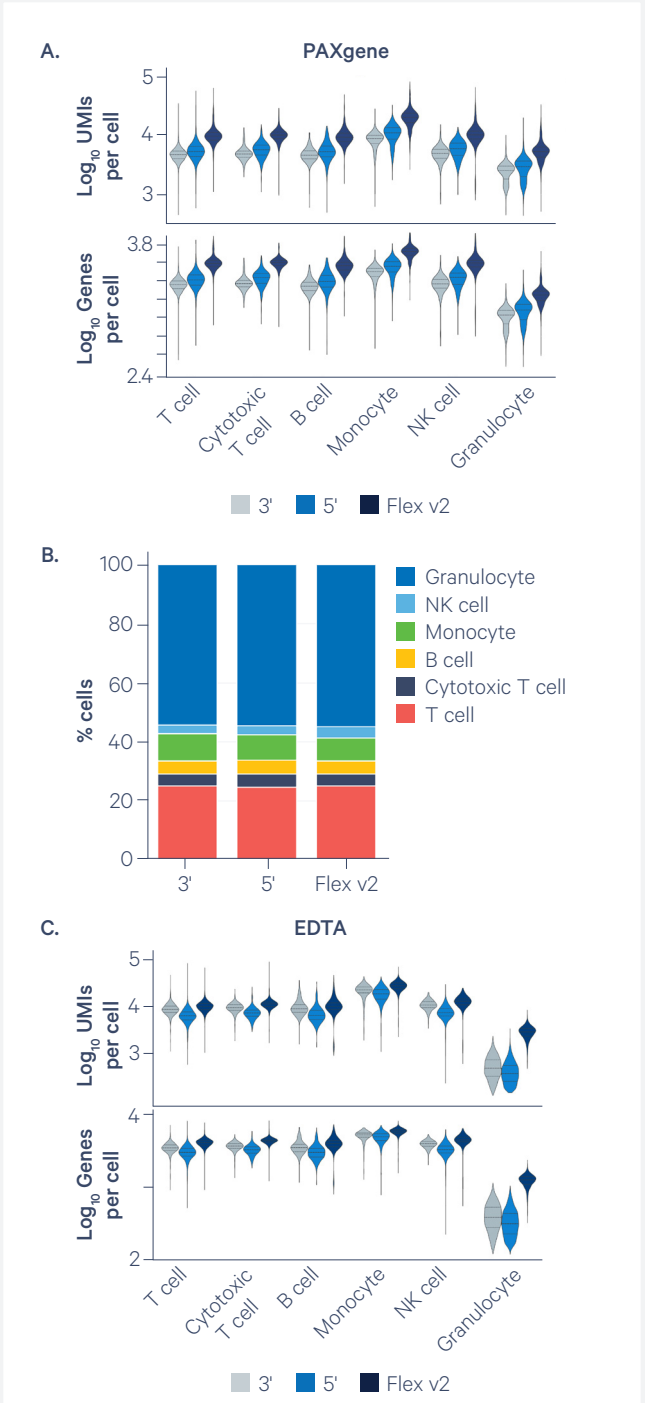


Figure 8. Leukocyte data from PAXgene vs. EDTA blood across GEM-X 3' v4, 5' v3, and Flex v2 assays. A. Log_{10} UMIs per cell and log_{10} genes per cell by cell type for PAXgene leukocytes. **B.** Immune cell type proportions for PAXgene leukocytes after removing RBCs post-sequencing. **C.** Log_{10} UMIs per cell and log_{10} genes per cell by cell type for EDTA leukocytes.

shipped in batches without fixation or other handling at the collection site, providing substantial logistical benefits for multi-site studies. In addition, PAXgene leukocyte isolation eliminates the need for bead-based RBC depletion, which simplifies handling and is valuable for high-throughput processing of large clinical cohorts. For PAXgene, keep storage temperature and duration consistent across all samples in a study, from blood draw through processing, to avoid introducing variability. Both 4°C and 20°C are supported for up to 3 days, with 4°C slightly favorable for GEM-X Flex v2 and GEM-X 5' v3 V(D)J.

Together, these two BCTs give researchers options matched to assay and cell type: Streck for PBMC-focused studies with GEM-X Flex v2, and PAXgene for leukocyte-focused gene expression and immune profiling where storage flexibility and frozen storage matter. Paired with 10x Genomics single cell assays, these tubes greatly expand the possibilities for single cell blood profiling in multi-site studies.

References

1. Chaudhary O, et al. Comprehensive cellular analysis with single-nucleus RNA-seq of archived PAXgene whole blood samples. *Cell Rep Methods* 6: 101333, 2026.
2. Blood Fixation and Cell Isolation for GEM-X Flex Gene Expression Demonstrated Protocol (CG000785)
3. GEM-X Flex v2 For Multiplexed Samples User Guide (CG000834)
4. Isolation of Leukocytes, Bone Marrow and Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing Demonstrated Protocol (CG000392)
5. Cell Surface & Intracellular Protein Labeling for GEM-X Flex Gene Expression Demonstrated Protocol (CG000781)
6. Fixation of Cells & Nuclei for GEM-X Flex Gene Expression Demonstrated Protocol (CG000782)
7. GEM-X Flex v2 with Feature Barcode technology for Protein User Guide (CG000835)
8. Chromium GEM-X Single Cell 5' v3 Gene Expression User Guide (CG000733)
9. Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols (CG000149)
10. Chromium GEM-X Single Cell 5' v3 Gene Expression with Feature Barcoding technology for Cell Surface Protein User Guide (CG000734)
11. Chromium GEM-X Single Cell 3' v4 Gene Expression User Guide (CG000731)

Appendix - Detailed Methods

Step 1: Blood Collection

Tube	Cell Type	Ideal Time Until Processing	Processing
Streck (Streck, 230469)	PBMCs	≤ 2 days at 4°C	Cell isolation or blood fixation
PAXgene (PreAnalytiX, 762165)	Leukocytes	≤ 3 days at 4°C or 20°C	Cell isolation or blood freeze
EDTA (BD, 366643)	PBMCs and leukocytes	≤ 2 hours at 4°C or 20°C	Cell isolation or blood fixation

Table 1. Blood collection tubes and storage windows

Step 2: Processing Workflows

In this Technical Note, processing refers to manual steps performed on stored blood. Blood from each tube type was processed using one of three workflows. Each workflow is defined by the order of fixation and cell isolation, and by the downstream 10x Genomics assay.

Workflow I. Blood Fixation First (Bead-Based Isolation)

Cell Type	1. Tube	2. Fixation and 3. Isolation	4. Assay
	GEM-X Flex v2 For Multiplexed Samples (CG000834)		
PBMCs	Streck or EDTA	CG000785 (2)	CG000834 (3)
Leukocytes	EDTA	CG000785 (2)	CG000834 (3)

Table 2. Blood in Workflow I is fixed first, then isolated for GEM-X Flex v2 assay

Workflow II. Cell Isolation First

Cell Type	1. Tube	2. Cell Isolation	3. Protein Labeling	4. Fixation	5. Assay/User Guide
PBMCs	**GEM-X Flex v2 Gene and Protein Expression**				
	Streck or EDTA	CG000392 (4), STEMCELL, 19654	CG000781 (5)	CG000782 (6)	CG000835 (7)
	GEM-X 5' v3 Gene Expression				
	EDTA	CG000392 (4), STEMCELL, 19654	-	-	CG000733 (8)
	GEM-X 5' v3 Gene and Protein Expression				
	EDTA	CG000392 (4), STEMCELL, 19654	CG000149 (9)	-	CG000734 (10)
Leukocytes	**GEM-X Flex v2 Gene Expression**				
	EDTA	CG000392 (4), STEMCELL, 18170	-	CG000782 (6)	CG000834 (3)
	GEM-X 5' v3 Gene Expression				
	EDTA	CG000392 (4), STEMCELL, 18170	-	-	CG000733 (8)
	GEM-X 3' v4 Gene Expression				
	EDTA	CG000392 (4), STEMCELL, 18170	-	-	CG000731 (11)

Table 3. Blood in Workflow II undergoes cell isolation first prior to processing for 10x single cell assays

Workflow III. PAXgene Leukocyte Isolation (Centrifugation-Based Wash)

Blood was stabilized directly in the PAXgene collection tube; no separate fixation step was required—including for GEM-X Flex v2. Blood was optionally stored at 4°C/20°C for up to 3 days before long-term storage at −80°C, then processed directly for leukocyte isolation prior to downstream assays. This Technical Note tested storage at 4°C and 20°C. Blood stored above 20°C may negatively impact data quality.

PAXgene Sample Freezing and Thawing

(Perform only if samples are frozen)

a. PAXgene blood can be frozen immediately after collection at −20°C. If long-term storage is preferred at −80°C, freeze blood overnight at −20°C before transferring to −80°C.

b. To thaw, place samples at 4°C overnight or 20°C for 2 hours. It is strongly recommended to keep the thawing temperature and duration consistent across the study. White precipitate may be visible at tube bottom when thawed. Mix the sample well before processing.

c. After thawing (4°C overnight or 20°C for 2 hours), incubate tubes at 20°C for an additional 2 hours before leukocyte isolation. This step is not needed if the blood was already held at 20°C for 2 hours before freezing.

Leukocyte Isolation: Reagents

Vendor	Item	Part Number
Corning	Phosphate-Buffered Saline, 1X without Calcium and Magnesium	21-040-CV
Millipore Sigma	Bovine Serum Albumin in DPBS (10%)	A1595
10x Genomics	RNase Inhibitor 40X	2001488 Kit PN-1000887
	Conc. Post-Hyb Buffer B	2001308 Kit PN-1000828*

**Additional tubes of Conc. Post-Hyb Buffer can be obtained from the GEM-X Flex Supplemental Wash Kit (PN-1000828)*

Leukocyte Isolation: Buffer Preparation

Prepare PAXgene Resuspension Buffer based on downstream assay.

For GEM-X Flex v2 with Probe Hybridization step to be performed in plates, prepare the plate-based formulation.

PAXgene Resuspension Buffer - Plate-Based (GEM-X Flex v2)
Prepare fresh; maintain on ice

	Stock	Final	Per Sample + 10% (µl)
PBS	-	-	2,026.2
BSA solution	10%	0.04%	8.8
RNase Inhibitor 40X	40X	1X	55.0
Conc. Post-Hyb Buffer B*	20X	1X	110.0
Total			**2,200.0**

**Improves sample retention in plate-based experiments.*

For GEM-X 5' v3, 3' v4, or Flex v2 with Probe Hybridization step to be performed in tubes, prepare the tube-based formulation.

PAXgene Resuspension Buffer - Tube-Based (GEM-X 5' v3, 3' v4, Flex v2)
Prepare fresh; maintain on ice

	Stock	Final	Per Sample + 10% (µl)
PBS	-	-	3,204.3
BSA solution	10%	0.04%	13.2
RNase Inhibitor 40X	40X	1X	82.5
Total			**3,300.0**

Leukocyte Isolation: Protocol Steps

a. Invert the PAXgene tube 3x. Transfer **1 ml** blood from the tube into a 2-ml round-bottom DNA LoBind tube.

b. Add **1 ml pre-chilled** PAXgene resuspension buffer, formulated as per the assay type (tube- or plate-based). See Buffer Preparation section.

c. Centrifuge at **500 g** for **5 min** at **4°C** using a swinging-bucket rotor.

d. Using a sterile plastic transfer pipette, remove the supernatant (dark red) without disturbing the pellet. Leave ≤ 10 µl behind for GEM-X Flex v2, or ≤ 30 µl behind for GEM-X 5' v3 and 3' v4.

TIPS *For GEM-X 3' v4, users can apply the CRISPR-based method for depleting RBC transcripts (1).*

e. **If performing GEM-X 5' v3 and 3' v4 assays**: Repeat steps b–d with another **1 ml pre-chilled** PAXgene resuspension buffer, for a total of two washes.

f. Add **1 ml pre-chilled** PAXgene resuspension buffer and pipette mix to resuspend the pellet.

g. Count cells by staining with propidium iodide (PI) or NucSpot 470. DO NOT use trypan blue for counting. It will count RBCs and lead to incorrect counts.

DO NOT dilute the isolated leukocyte stock sample.

TIPS *If hemoglobin causes high background and reduces counting accuracy, dilute an aliquot of the sample 1:2–1:4 in 1X PBS for counting purposes only.*

h. After leukocyte isolation, keep samples on ice for up to 4 h before GEM loading (GEM-X 3' v4 and 5' v3) or probe hybridization (GEM-X Flex v2) if more time is needed for cell counting. Storing isolated leukocytes at −80°C is not supported.

TIPS *For GEM-X Flex v2, after the Post-Hybridization Washes, PAXgene leukocyte samples can be stored at −80°C for up to 1 month.*

Leukocyte Isolation: Downstream Processing

After leukocyte isolation, one of the following protocols was performed:

- GEM-X Flex v2 For Multiplexed Samples (CG000834)
- Chromium GEM-X Single Cell 3' v4 Gene Expression (CG000731)
- Chromium GEM-X Single Cell 5' v3 Gene Expression (CG000733)

Document Revision Summary

Document Number	CG001727
Title	Extended Collection-to-Processing Windows with Blood Collection Tubes for 10x Single Cell Assays
Revision	Rev A
Revision Date	October 2026
Description of Changes	• Initial release of document

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