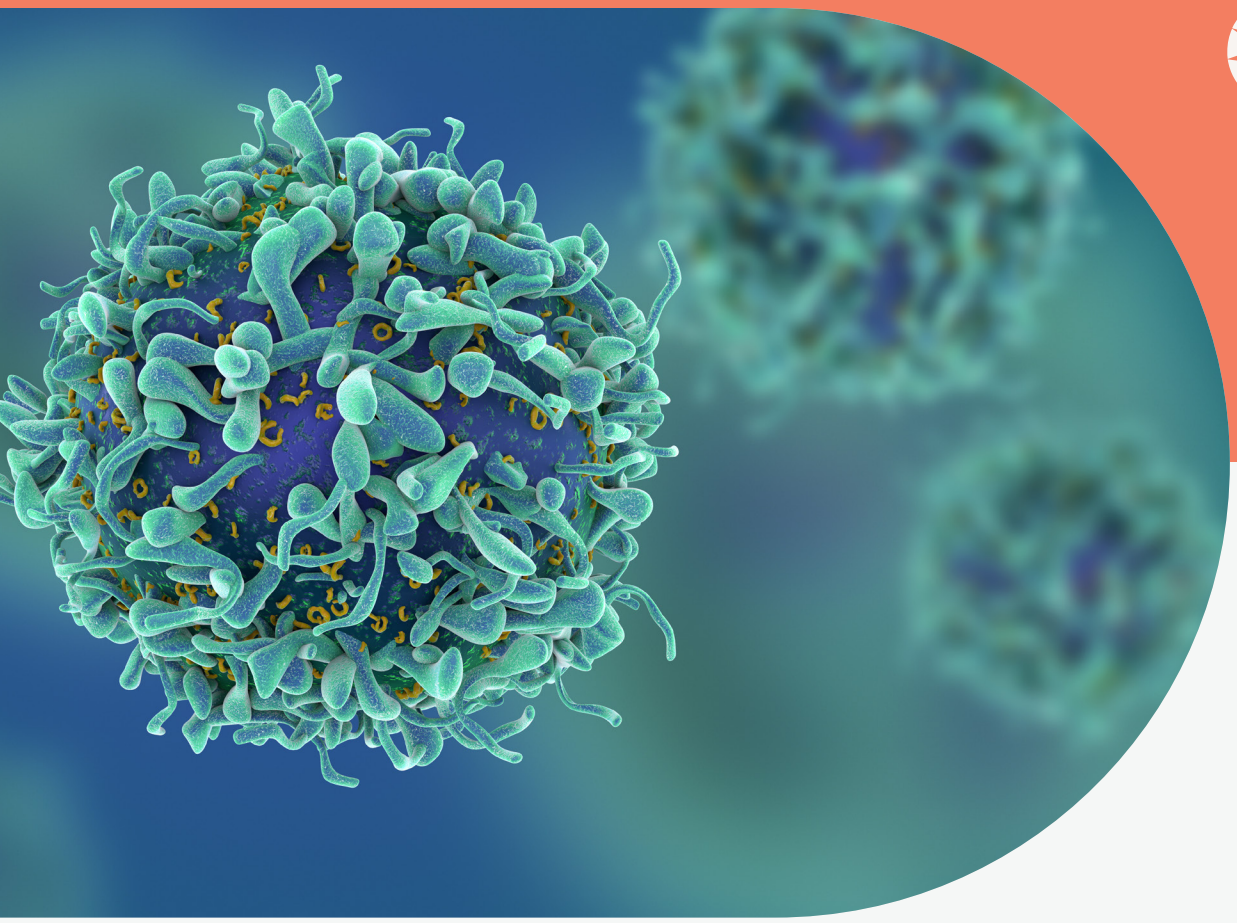

# BD® OMICS-Guard Sample Preservation Buffer

## Protect your samples, guard your science

- Stress-free, one-step preservation protocol with minimum hands-on time
- Optimized to preserve cells for a variety of downstream transcriptomic, proteomic and multiomic applications including RNA-seq, CITE-seq, flow cytometry and qPCR
- Protects cell viability and preserves different cell populations in your samples for up to 72 hours at 4 °C
- Developed and tested across multiple sample types: PBMCs and tissue samples
- Available in two, easy-to-use formats: 50-mL bottle or 12 x 1-mL vials

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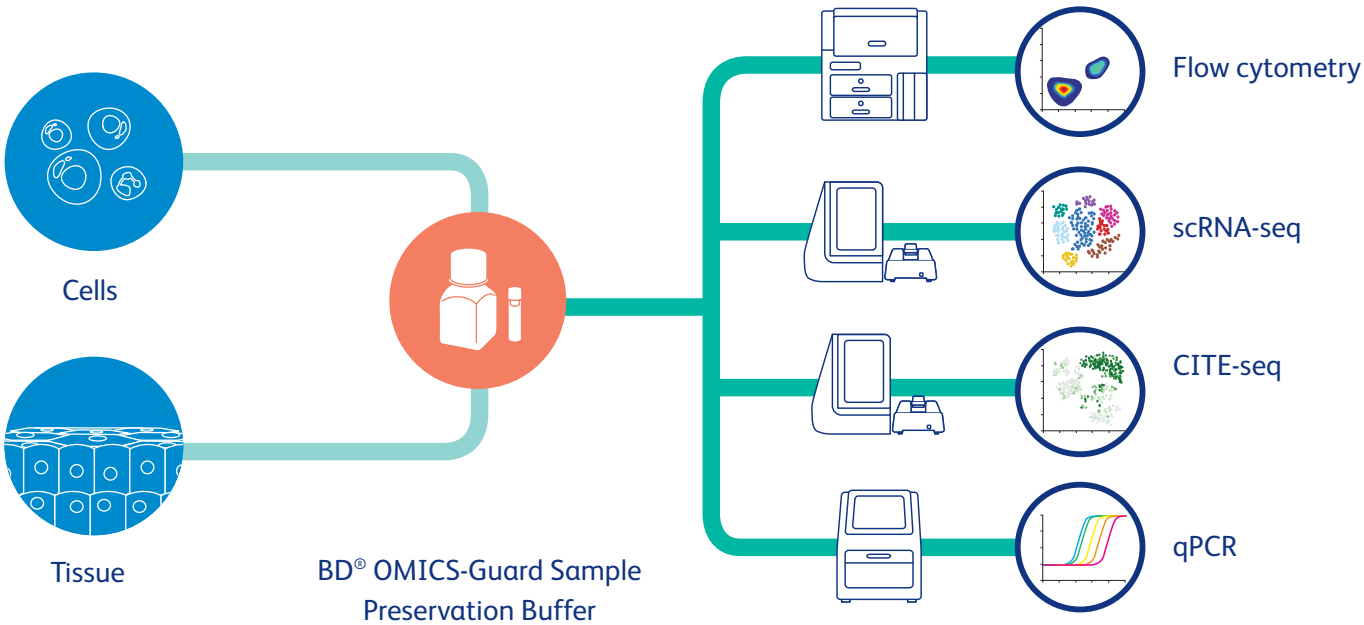
part of Thermo Fisher Scientific

Being able to preserve biological samples is critical, especially for research involving multi-site studies and collaborations, where samples are often collected at different times and locations. The viability and integrity of preserved samples are of paramount importance while preserving biological samples. Recognizing this need, BD has developed the BD® OMICS-Guard Sample Preservation Buffer.

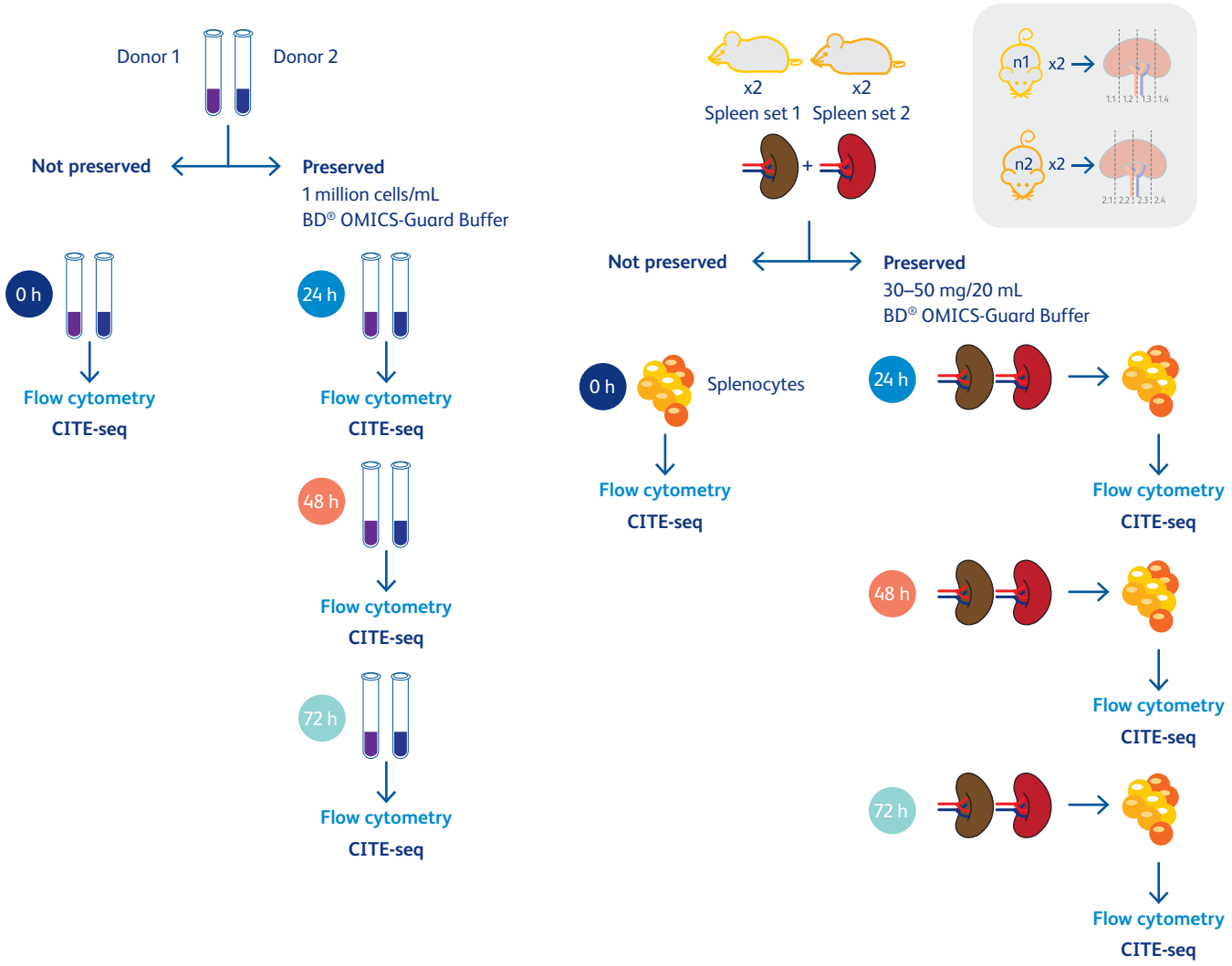
Here, we present a trio of illustrative datasets showing the versatile applications of the BD® OMICS-Guard Buffer. These datasets encompass (1) a comprehensive CITE-seq experiment with two different sample types conducted on the BD Rhapsody™ Single-Cell Analysis System; (2) utilization of the BD® OMICS-Guard Buffer to preserve protein epitopes for flow cytometry analyses; and (3) detailed qPCR analyses.




## 1 CITE-seq analyses with BD® OMICS-Guard Buffer-preserved samples

CITE-seq analyses were conducted on the BD Rhapsody™ Single-Cell Analysis System with PBMCs and tissues preserved in BD® OMICS-Guard Buffer. Cell viability, 3' gene expression, surface protein expression and cell populations in both human PBMCs and mouse spleen tissues were analyzed and compared to non-preserved samples (controls) in a time-course study over 72 hours. High concordance of transcriptomic and proteomic profiles and consistency in cell population frequencies were found between the control and preserved samples.

A.




B. BD® AbSeq Immune Discovery Panel used for human PBMC analyses

| Specificity | Clone |
|---|---|
| CD11c | B-Ly6 |
| CD14 | MPHIP9 |
| CD185 (CXCR5) | RF8B2 |
| CD19 | SJ25C1 |
| CD25 | 2A3 |
| CD27 | M-T271 |
| CD278 | DX29 |
| CD279 | EH12.1 |
| CD3 | UCHT1 |
| CD357 (GITR) | V27-580 |
| CD366 (Tim3) | 7D3 |
| CD4 | SK3 |
| CD45RA | HI100 |
| CD56 | NCAM16.2 |
| CD62L | DREG-56 |

| Specificity | Clone |
|---|---|
| CD197 (CCR7) | 2-L1-A |
| CD186 (CXCR6) | 13B 1E5 |
| CD127 | HIL-7R-M21 |
| CD134 | ACT35 |
| CD28 | L293 |
| CD272 | J168-540 |
| CD8 | SK1 |
| HLA-DR | G46-6 |
| CD16 | 3G8 |
| CD183 | 1C6/CXCR3 |
| CD196 (CCR6) | 11A9 |
| CD137 | 4B4-1 |
| CD161 | HP-3G10 |
| IgM | G20-127 |
| IgD | IA6-2 |

BD® AbSeq Antibody-Oligos used for mouse spleen analyses

| Cat. No. | Specificity | Clone | Cat. No. | Specificity | Clone | Cat. No. | Specificity | Clone |
|---|---|---|---|---|---|---|---|---|
| 940135 | IgM | II/41 | 940111 | CD19 | 1D3 | 940184 | CD162 | 2PH1 |
| 940179 | CD49d | R1-2 | 940008 | CD11b | M1/70 | 940164 | H-2Kb | AF6-88.5 |
| 940198 | CD115 | T38-320 | 940414 | CD141 | LS17-9 | 940411 | CD319 CRACC | 4G2 |
| 940186 | CD106 | 429 MVAM.A | 940131 | F4/80 | T45-2342 | 940158 | CD29 | HM B1-1 |
| 940120 | CD28 | 37.51 | 940330 | CD268 BAFF-R | 7H22-E16 | 940110 | CD45R/B220 | RA3-6B2 |
| 940200 | CD9 | KMC8 | 940410 | Siglec-H | 440c | 940119 | Ly6G & Ly-6C | RB6-8C5 |
| 940321 | CD11c | N418 | 940498 | CD195 | C34-3448 | 940333 | CD3 | 17A2 |
| 940334 | Vγ1.1 TCR | 2.11 | 940483 | CD107b | M3/84 | 940471 | CD4 | GK1.5 |
| 940108 | CD4 | RM4-5 | 940446 | CD47 | miap301 | 940459 | CD180 | RP/14 |
| 940345 | CD8a | 53-6.7 | 940145 | CD43 | S7 | 940320 | CD45 | 30-F11 |

**Figure 1. Overview of experimental design.**

**A**) Time course experiments using previously frozen human PBMCs (left) and fresh mouse spleen tissue (right). Human PBMCs from two donors were thawed and a portion from each donor was not preserved and used as controls on Day 0 (0 h). The rest of the PBMCs were preserved with BD® OMICS-Guard Buffer at 1 million cells/mL buffer. Spleen tissue was harvested from C57/BL6 mice and equally proportioned into four pieces, three of which were preserved in BD® OMICS-Guard Buffer at 30–50 mg tissue/20 mL buffer. A piece of mouse spleen tissue from each mouse was not preserved and used as control on Day 0 (0 h). The hPBMCs and mouse spleen tissues were stored in BD® OMICS-Guard Buffer for a total of 72 h at 4 °C. At each time point, 24 h, 48 h and 72 h, a portion of preserved PBMCs and tissue were removed, split for flow cytometry analysis and single-cell multiomics analysis on the BD Rhapsody™ Single-Cell Analysis System, and compared to controls at 0 h. For single-cell multiomics analysis on the BD Rhapsody™ Single-Cell Analysis System, hPBMCs were co-stained with the BD® AbSeq Immune Discovery Panel (IDP) and BD® Human Single-Cell Multiplexing Kit (hSMK) and pooled before single-cell capture. Mouse splenocytes were extracted from preserved mouse spleen tissues, co-stained with a 30-plex mouse BD® AbSeq Ab-Oligo Panel and BD® Single-Cell Multiplexing Set Rat Anti-Mouse MHC-H2 Class I (M1/42), and pooled before cell viability quantification. Following single-cell capture on the BD Rhapsody™ System, whole transcriptome analysis (WTA), AbSeq and SMK libraries were generated from subsampled beads at 4,000 cells. The BD Rhapsody™ Sequence Analysis Pipeline was used for single-cell multiomics data analysis. **B**) Top table: 30-plex BD® AbSeq IDP used for human PBMC staining. Bottom table: 30-plex mouse BD® AbSeq Panel used for mouse splenocyte staining.

## BD® OMICS-Guard Buffer preserves cell viability in single-cell and tissue samples

Cell viability is one of the pivotal factors in cell-based assays in any single-cell assay. Common sample preservation methods such as freezing can result in high cell death after thawing and can also be challenging for shipping. BD® OMICS-Guard Buffer helps maintain cell viability for up to 72 hours (>70% for both PBMC and tissue samples) via a simple and gentle preservation protocol.

| | Human PBMC viability (%) | |
|---|---|---|
| Time point | Donor 1 | Donor 2 |
| 0 h | 96.25 | 95.21 |
| 24 h | 83.17 | 86.81 |
| 48 h | 79.18 | 78.35 |
| 72 h | 77.63 | 76 |

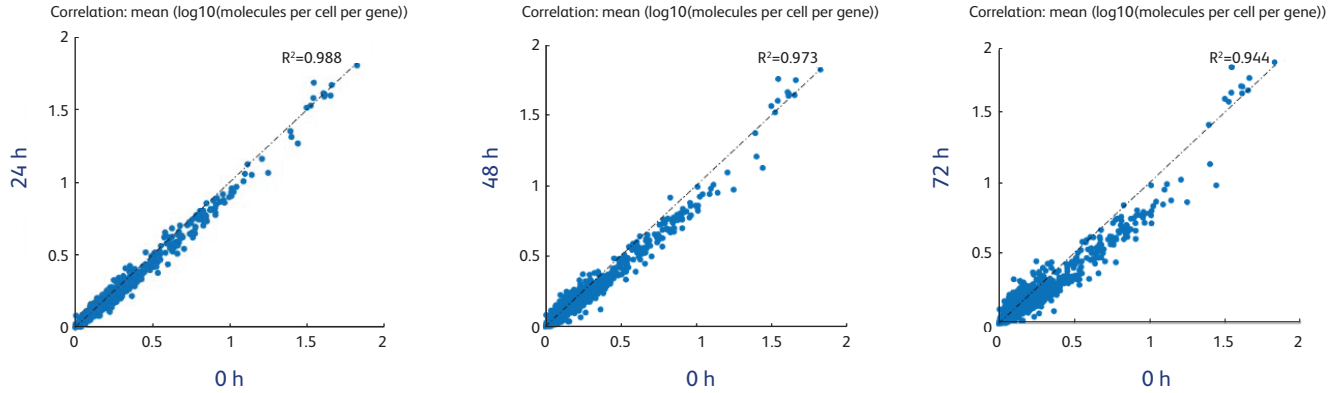
**Figure 2. Cell viability of preserved samples.**

Human PBMCs, both controls and BD® OMICS-Guard Buffer-preserved samples were stained with DRAQ7™ and Calcein AM and the cell viability metric was quantified with the BD Rhapsody™ Scanner.

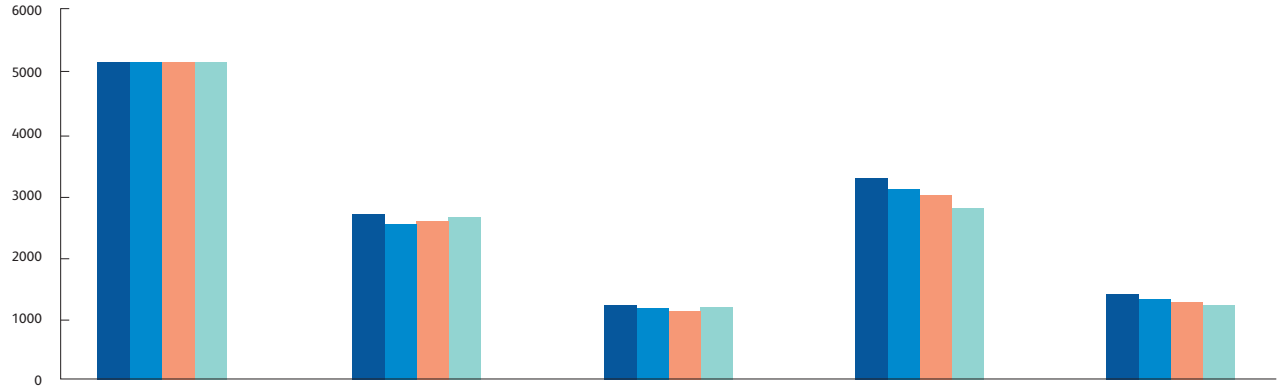
# BD® OMICS-Guard Buffer preserves transcriptome profiles in single-cell and tissue samples

BD® OMICS-Guard Sample Preservation Buffer preserves cellular transcriptomic integrity and expression profiles for up to 72 hours at 4 °C. In this CITE-seq study, gene expression of single cells was profiled using the BD Rhapsody™ WTA Assay. Results showed high concordance of single-cell gene expression and WTA sensitivity (gene and molecule counts) over the 72-h storage period, indicating that transcriptomic information was well preserved with the BD® OMICS-Guard Buffer.

A.

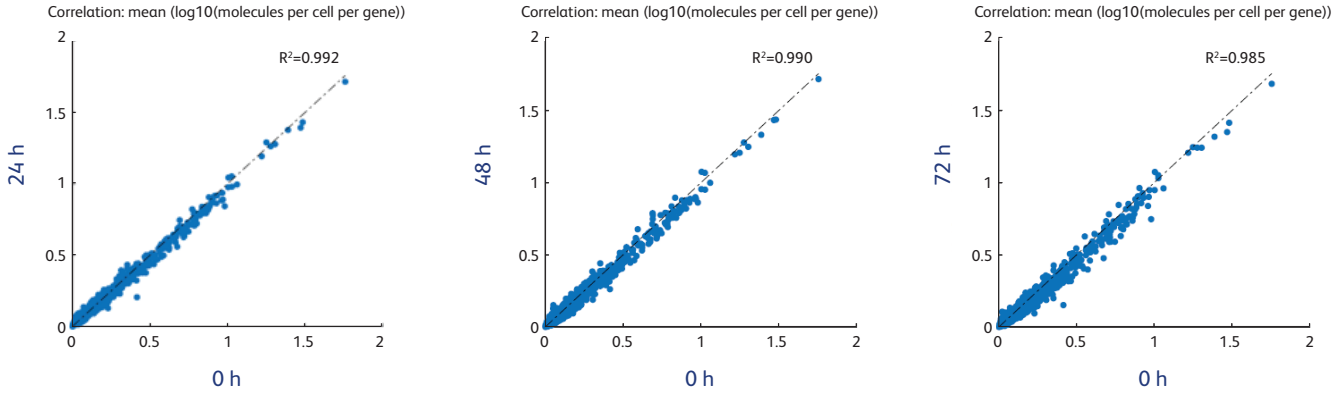



B. Human PBMC WTA assay sensitivity

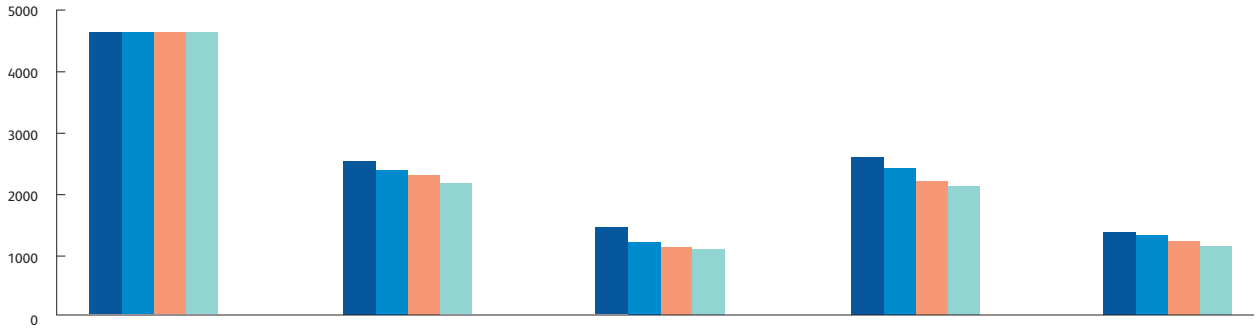



| | Donor 1 and Donor 2 Mean reads per cell | Donor 1 Median molecules/cell | Donor 1 Median genes/cell | Donor 2 Median molecules/cell | Donor 2 Median genes/cell |
|---|---|---|---|---|---|
| 0 h | 5211 | 2712 | 1222 | 3285 | 1402 |
| 24 h | 5200 | 2567 | 1159 | 3122 | 1335 |
| 48 h | 5204 | 2608 | 1144 | 3039 | 1294 |
| 72 h | 5206 | 2663 | 1148 | 2841 | 1203 |

C.



D. Mouse splenocyte WTA assay sensitivity




| | Sample 1 and 2 Mean reads per cell | Sample 1 Median molecules/cell | Sample 1 Median genes/cell | Sample 2 Median molecules/cell | Sample 2 Median genes/cell |
|---|---|---|---|---|---|
| 0 h | 4676.49 | 2558 | 1486 | 2631 | 1387 |
| 24 h | 4673.11 | 2391 | 1300 | 2455 | 1319 |
| 48 h | 4672.62 | 2336 | 1254 | 2202 | 1221 |
| 72 h | 4680.95 | 2181 | 1171 | 2150 | 1155 |

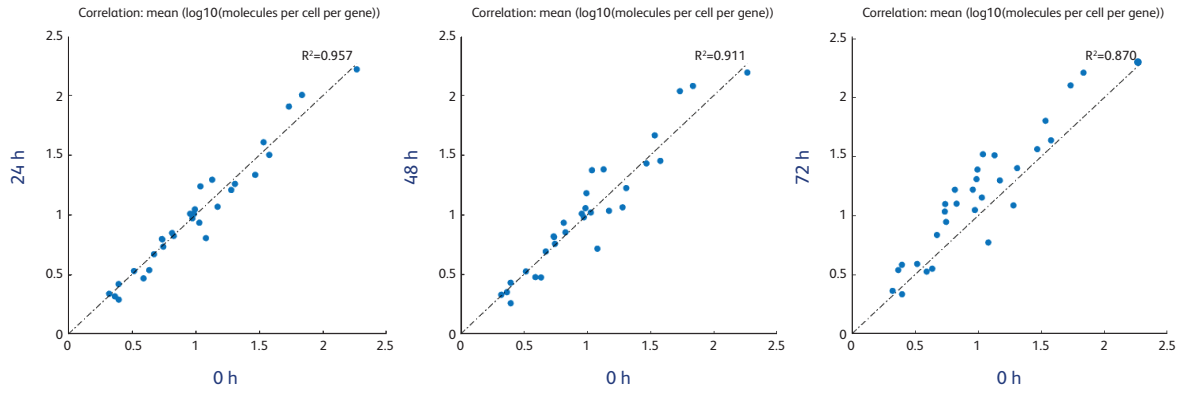
**Figure 3. Gene expression and WTA sensitivity are in high concordance over the 72-h storage period.**

**A and C)** Gene expression correlation between control samples (0 h) and preserved samples at 24 h, 48 h and 72 h in hPBMCs (**3A**: donor 1, $R^2$>0.9) and mouse splenocytes (**3C**: sample 1, $R^2$>0.9). $R^2$ correlation values were calculated using differentially expressed gene plots from WTA data on the BD Rhapsody™ System, for human PBMCs (**3A**) and mouse splenocytes (**3C**). **B and D**) WTA assay sensitivity represented by median molecules per cell (median transcripts per cell) and median genes per cell were compared between control samples (0 h) and preserved samples at 24 h, 48 h and 72 h in human PBMCs (**3B**) and mouse splenocytes (**3D**). Sequencing data were normalized to the same read-depth and samples were demultiplexed.

## BD® OMICS-Guard Buffer preserves proteome profiles in single-cell and tissue samples

BD® OMICS-Guard Sample Preservation Buffer preserves proteomic integrity and expression profiles for up to 72 hours at 4 °C. Single-cell surface protein expression was quantified using a 30-plex BD® AbSeq Ab-Oligo Panel. High protein expression correlation and comparable AbSeq sensitivity was observed between the control and preserved samples.

A.



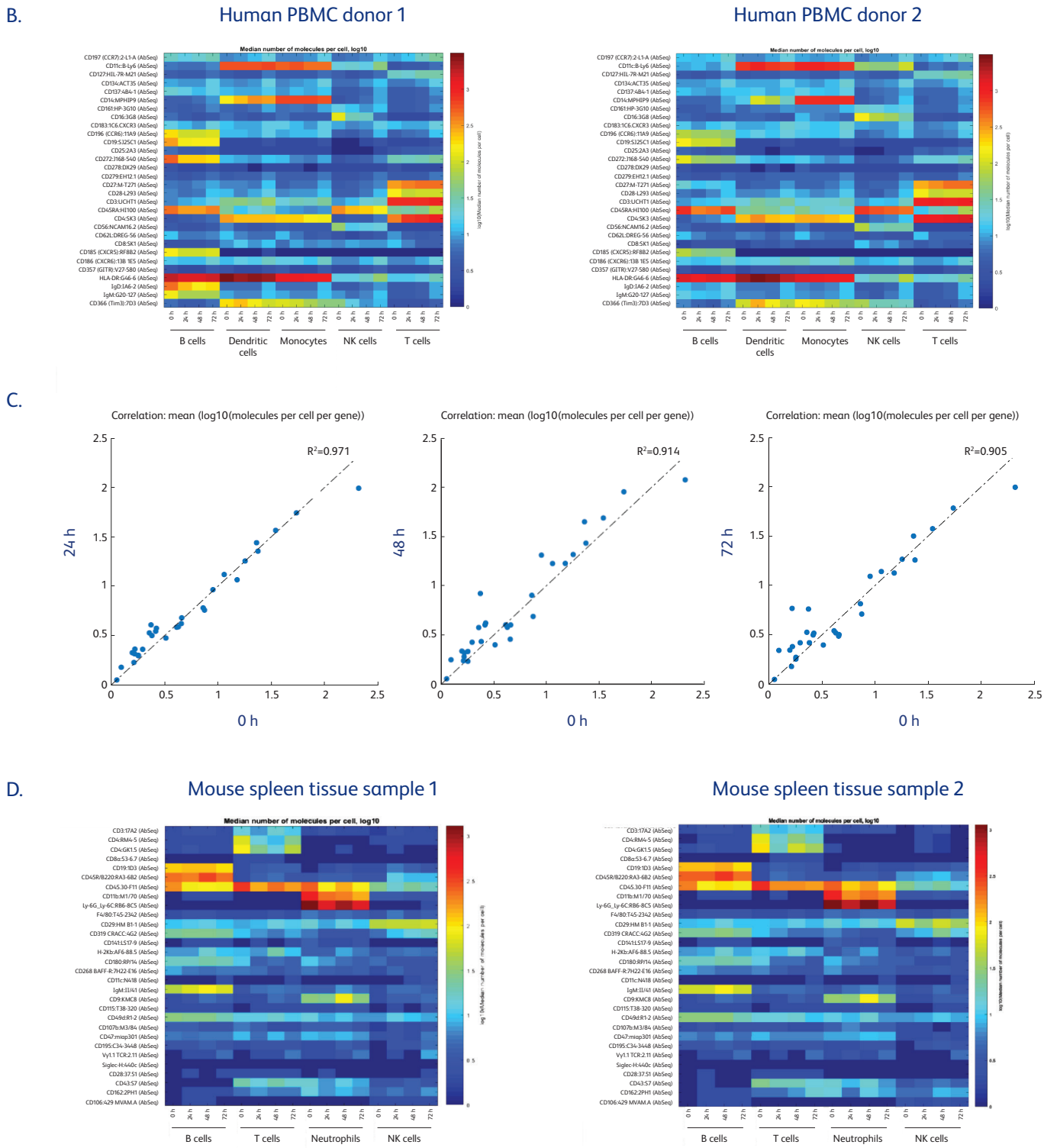



**Figure 4. AbSeq expression and sensitivity are in high concordance over the 72-h storage period.**

**A and C)** AbSeq signal representing protein expression between control samples (0 h) and preserved samples at 24-h, 48-h and 72-h time points in hPBMCs (**4A**: donor 1, $R^2$>0.8) and mouse splenocytes (**4C**: sample 1, $R^2$>0.9). Sequencing data were normalized to the same read-depth followed by demultiplexing of samples. **B and D**) AbSeq sensitivity represented by median molecules per cell of each BD® AbSeq Ab-Oligo used to stain human PBMCs (**4B**) and mouse splenocytes (**4D**) in major PBMC cell types (B, T, NK, dendritic cells and monocytes) and major splenic cell types (B, T, NK cells and neutrophils), respectively. Cell type annotation and time points (0, 24, 48 and 72 h) are shown to facilitate specificity performance of AbSeq in preserved cells. Comparable protein expression is found over the 72-h storage period.

## Cell subpopulation frequencies of single-cell and tissue samples are preserved by BD® OMICS-Guard Buffer

Impact to specific cell populations during sample storage could compromise analysis results and conclusions. BD® OMICS-Guard Sample Preservation Buffer ensures recovery of major cell populations and preserves the frequency of each cell population in different sample types during storage at 4 °C.

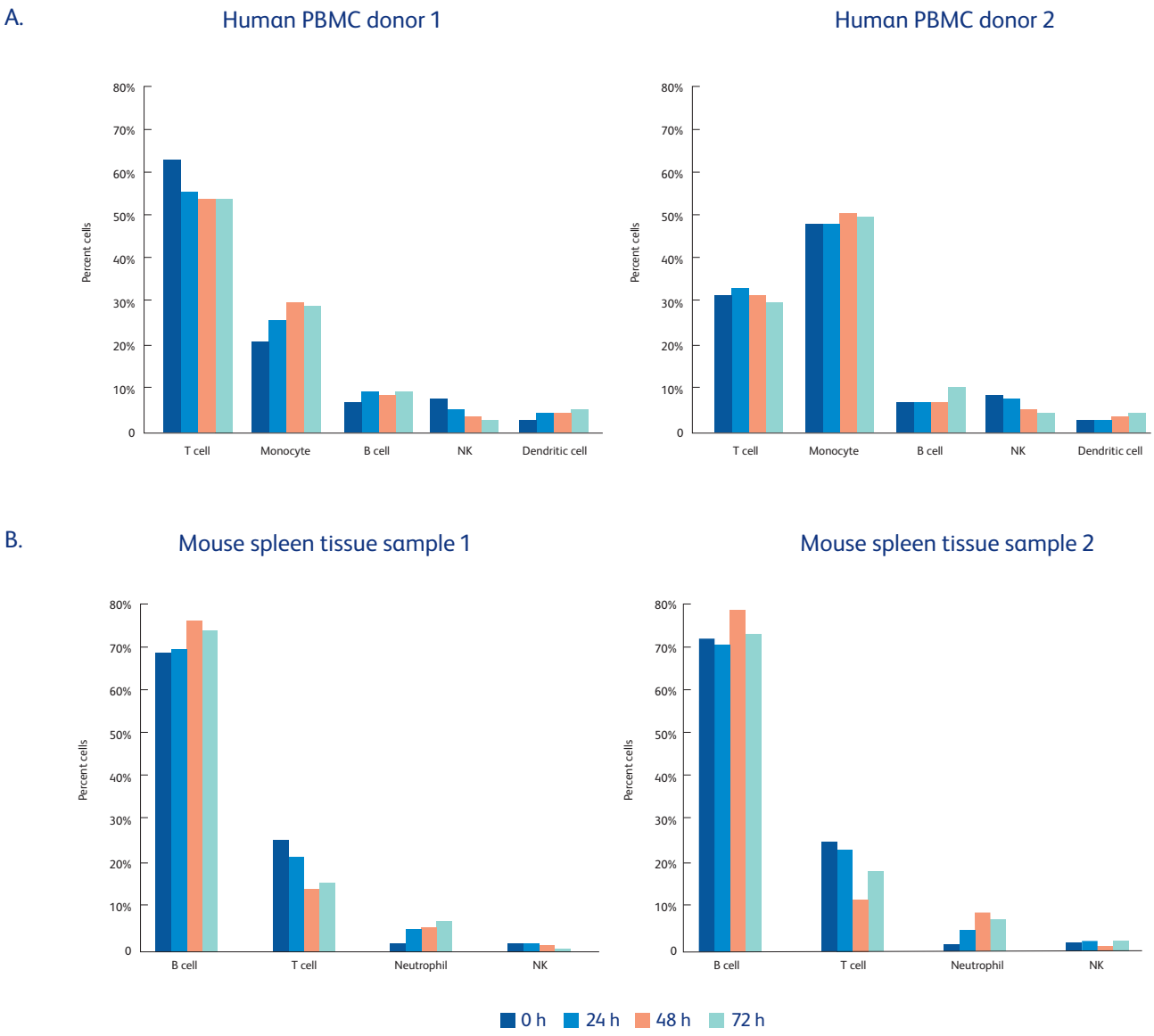



**Figure 5. Frequencies of major cell populations.**

The frequencies of major cell populations in hPBMCs (**5A**) and mouse splenocytes (**5B**) were maintained by BD® OMICS-Guard Buffer preservation across different time points (24, 48, 72 h) compared to control samples. Cell type annotation of hPBMCs was based on the immune cell type caller embedded in the BD Rhapsody™ Sequence Analysis Pipeline. Cell types in mouse spleen tissue were manually annotated based on gene expression.

# 2 Proteomic profiling of BD® OMICS-Guard Buffer-preserved samples using flow cytometry analyses

Human PBMC samples and mouse spleen tissues from the aforementioned CITE-seq experiments (Figure 1A) were analyzed by flow cytometry for surface protein expression profiling.

A.

| Human 13-plex flow panel | | |
|---|---|---|
| Specificity | Dye | Clone |
| CD45 | BUV395 | HI30 |
| CD56 | BUV615 | NCAM16.2 |
| CD4 | BUV805 | SK3 |
| CD19 | BV421 | HIB19 |
| CD3 | BV480 | UCHT1 |
| CD8 | BV605 | SK1 |
| CD16 | BV650 | 3G8 |
| CCR7 | BV786 | 2-L1-A |
| CD45RO | BB515 | UCHL1 |
| CD14 | PE-CF594 | MϕP9 |
| CD25 | RB780 | 2A3 |
| CD127 | Alexa Fluor™ 647 | HIL-7R-M21 |
| FVS780 (APC-Cy7 channel) | | |

B.

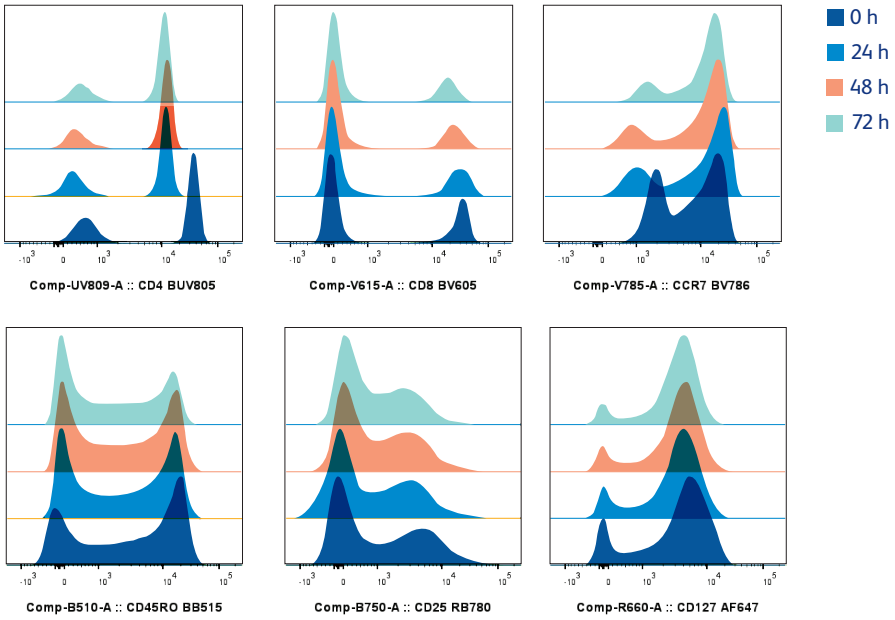
| Mouse 11-plex flow panel | | |
|---|---|---|
| Specificity | Dye | Clone |
| CD3 | BUV737 | 17A2 |
| CD4 | BV650 | GK1.5 |
| CD8a | BV605 | 53-6.7 |
| CD19 | PE-CF594 | 1D3 |
| CD11c | BV421 | HL3 |
| CD45 | PE-Cy7 | 30-F11 |
| Ly6-G/C | R718 | RB6-8C5 |
| CD44 | BUV395 | IM7 |
| CD43 | PE | S7 |
| CD62L | APC | MEL-14 |
| CD49b | FITC | DX5 |

**Figure 6. Overview of flow cytometry experiments.**

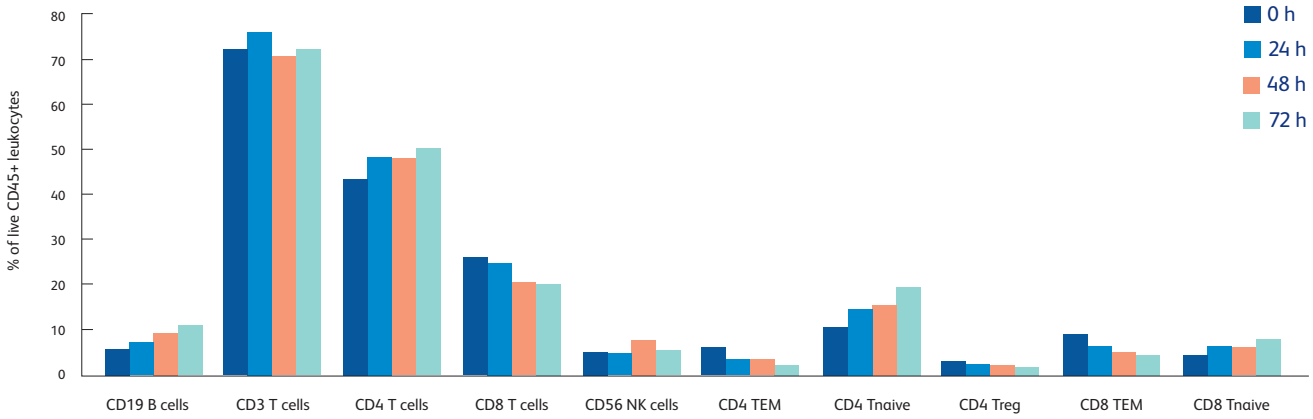
Human PBMCs and mouse splenocytes were obtained as described in **Figure 1** and analyzed by flow cytometry. **A)** Human PBMCs were stained with a 13-color fluorescent panel for major immune cell population identification and protein expression analysis. **B)** Mouse splenocytes were stained with an 11-color fluorescent panel for major immune cell population identification and protein expression analysis. For both hPBMCs and mouse splenocytes, fluorescent antibodies were stained at optimal concentrations and labeling volumes in BD Pharmingen™ Stain Buffer.

The protein expression level and frequencies of major cell populations revealed by flow cytometry analyses in both human PBMCs and mouse splenocytes stayed consistent over the course of 72 hours, indicating that the BD® OMICS-Guard Buffer can preserve surface protein epitopes for single-cell proteomics analyses and protect the cell composition of single-cell or tissue samples.

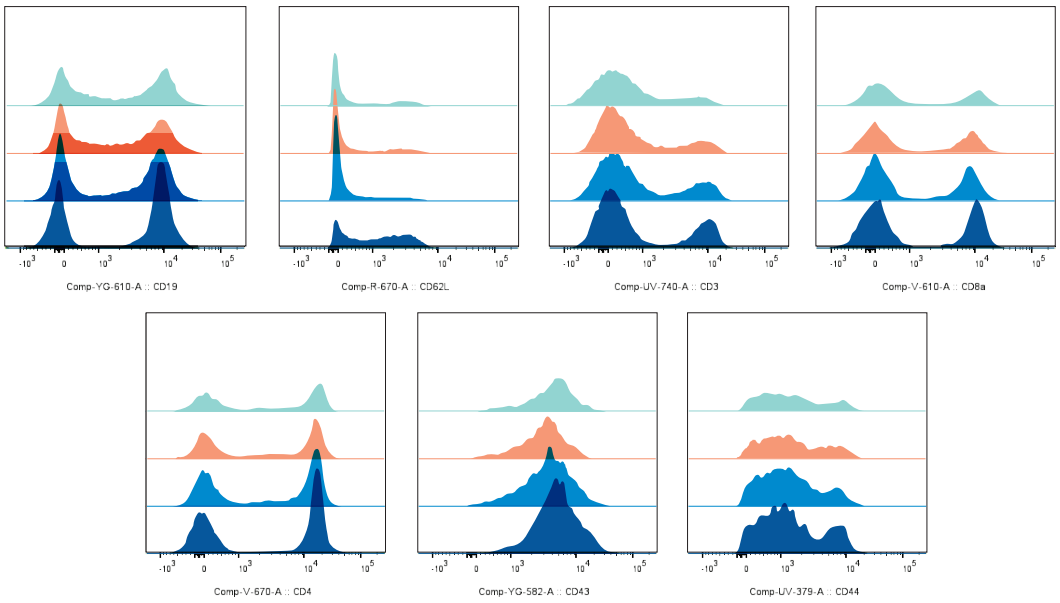
A.



B.




C.




D.

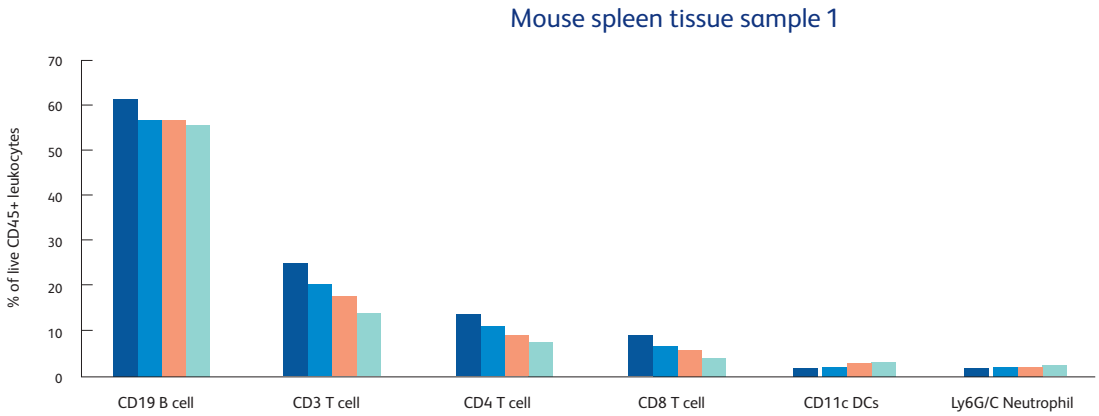


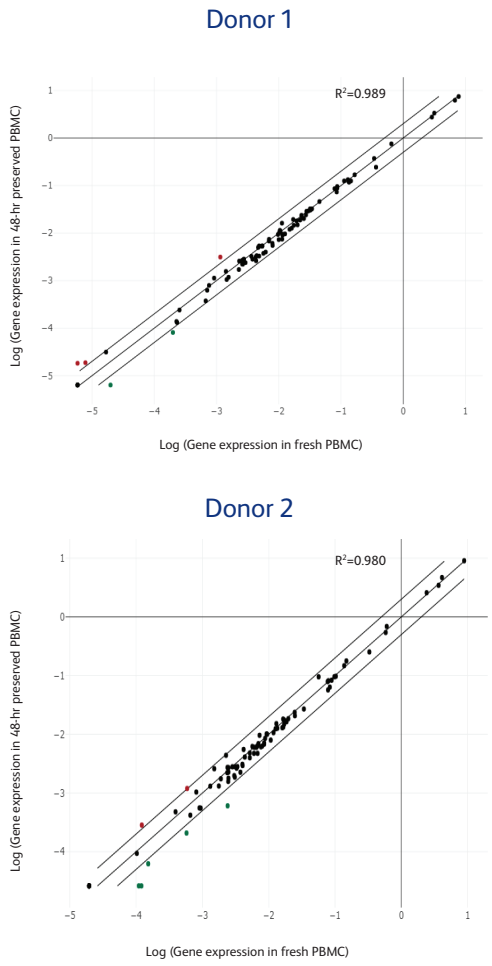

**Figure 7. Proteomic profiles and major immune cell population frequencies of samples revealed by flow cytometry.**

**A and C)** Flow histogram plots show consistent key protein marker expression represented by fluorescent signal between control (0 h) and preserved samples over time (24, 48, 72 h) for human PBMC (**7A**, donor 1) and mouse spleen samples (**7C**, Sample 1). For human PBMCs, CD14+ and CD16+ cells were excluded from live CD45+ leukocytes. NK cells and B cells were identified from CD14-CD16- cells. CD4+ and CD8+ T cells were gated from CD3+ T cells. Effector memory T cells and regulatory T cells were evaluated on CD4+ T cells. For mouse spleen tissue, CD19 and CD3 were gated on CD45+; CD62L was gated on CD19-. CD4 and CD8a were gated on CD3+. Additionally, we show signal histograms for lymphocyte surface proteins CD43 and CD44 in CD4 event clusters. **B and D)** The frequencies of different CD45+ immune cell populations or major splenic leukocyte cell populations stay consistent over the time for human PBMC and mouse spleen tissue samples, respectively.

# 3 Gene expression analysis using qPCR with BD® OMICS-Guard Buffer-preserved samples

Storage conditions often induce cellular stress. In a separate set of experiments, to evaluate the impact of BD® OMICS-Guard Sample Preservation Buffer on cellular stress pathways, a qPCR study was performed wherein the expression of stress-associated genes in fresh and BD® OMICS-Guard Buffer-preserved PBMC samples was compared. Results revealed no significant expression change of stress-associated genes after 48 h of preservation. Our data demonstrate minimum cellular stress upon preservation with BD® OMICS-Guard Buffer.

A.




B. Fold change (48-h preserved sample vs fresh PBMCs)

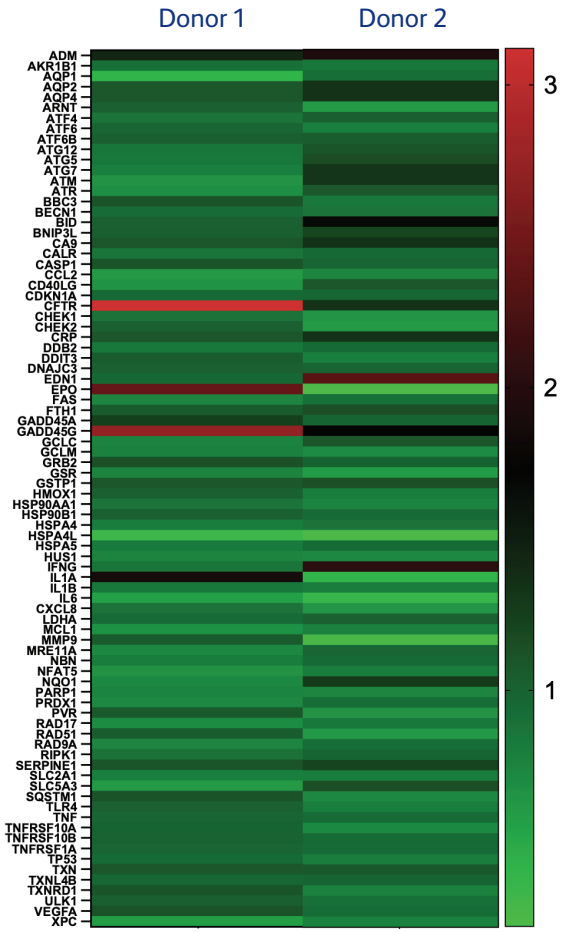



**Figure 8. Gene expression analysis using qPCR.**

RNA was isolated from fresh and 48-h preserved human PBMCs from two donors using the RNeasy™ Mini Kit (Qiagen). RIN (RNA integrity number) was evaluated using an Agilent TapeStation System after RNA isolation. cDNA was produced using the RT2™ First Strand Kit (Qiagen), and bulk gene expression of 84 stress-associated genes was analyzed using the RT² Profiler™ PCR Array (Human Stress and Toxicity PathwayFinder; Qiagen). **A)** Comparable expression of 84 stress-associated genes in fresh and 48-h preserved PBMCs (gene expression correlation $R^2$>0.9 in PBMCs from both donors). **B)** Heatmap showing low fold-change of expression of stress-associated genes in preserved PBMC samples compared to that of fresh PBMCs.

## Materials used in these studies

| | |
|---|---|
| BD® OMICS-Guard Sample Preservation Buffer Kit | 570908 |
| BD® OMICS-Guard Sample Preservation Buffer | 570911 |
| BD Rhapsody™ Scanner | 633701 |
| BD Rhapsody™ HT Xpress System Package | 666625 |
| BD® AbSeq Enhancer Kit | 570750 |
| BD Rhapsody™ Enhanced Cartridge Reagent Kit | 664887 |
| BD Rhapsody™ 8-Lane Cartridge | 666262 |
| BD Rhapsody™ cDNA Kit | 633773 |
| BD Rhapsody™ Whole Transcriptome Analysis (WTA) Amplification Kit | 633801 |
| BD® AbSeq Immune Discovery Panel | 625970 |
| BD® Human Single-Cell Multiplexing Kit | 633781 |
| BD® Single-Cell Multiplexing Set Rat Anti-Mouse MHC-H2 Class I (M1/42) | 626545 |
| BD Pharmingen™ Human Fc Block™ Reagent | 564219 |
| BD Pharmingen™ Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™ Reagent) | 553141 |
| BD Pharm Lyse™ Lysing Buffer | 555899 |
| BD Pharmingen™ Stain Buffer (FBS) | 554656 |

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