

# BD<sup>®</sup> 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

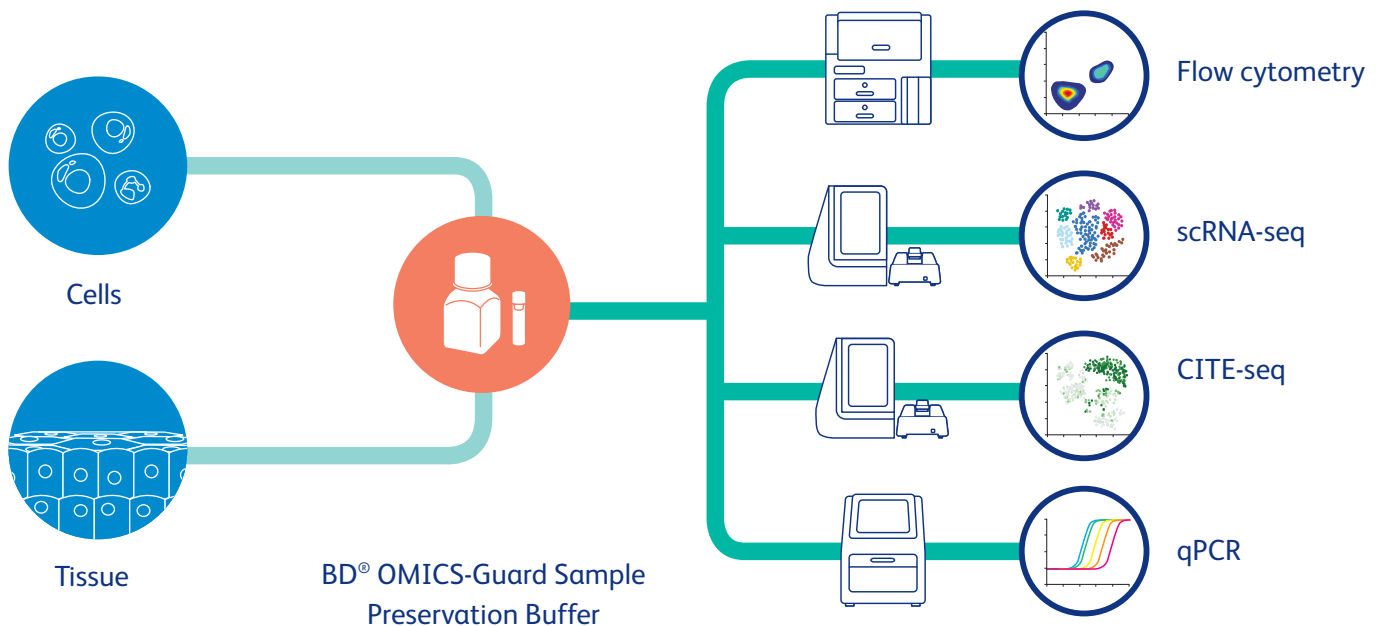


Available in two, easy-to-use formats: 50-mL bottle or 12 x 1-mL vials



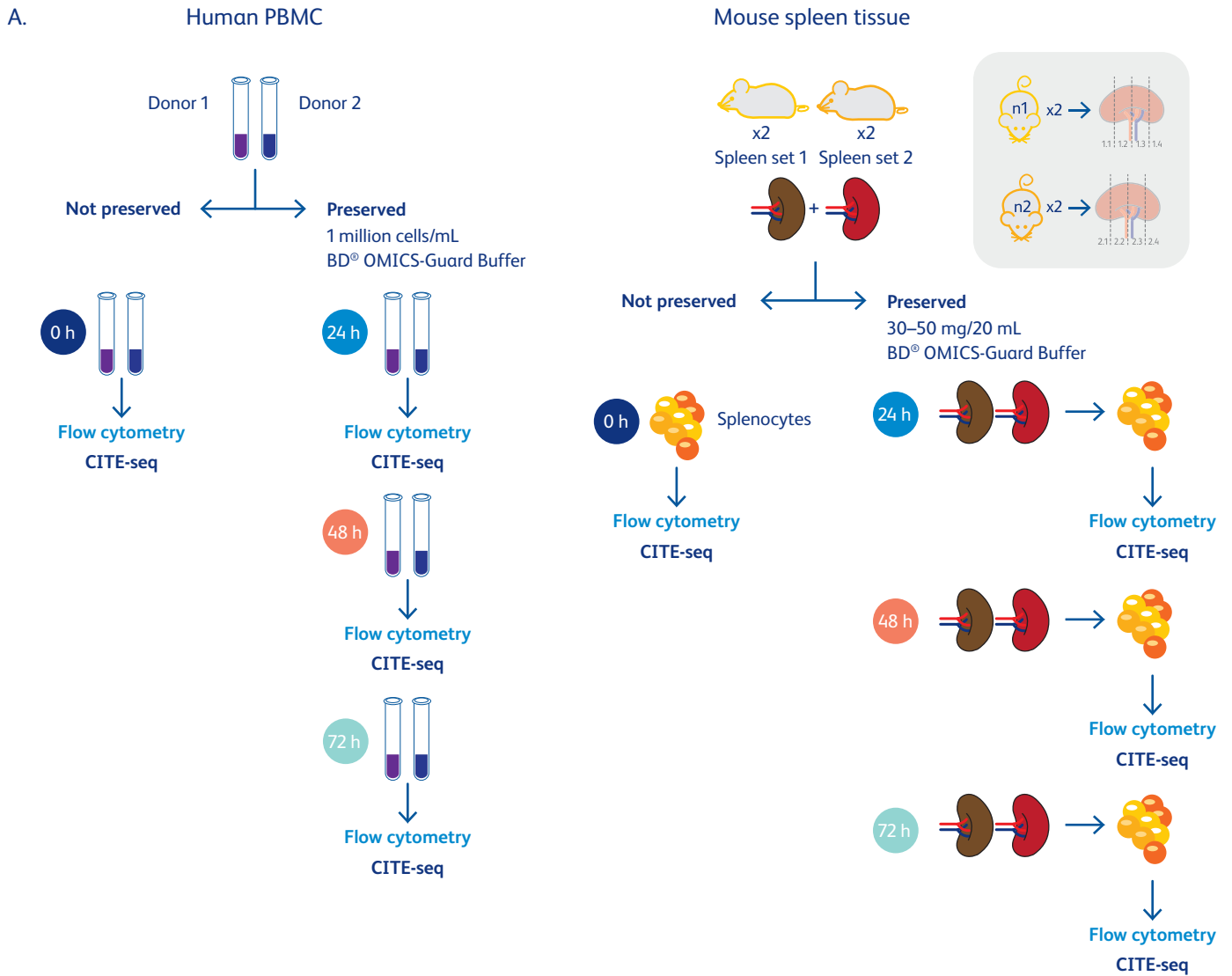
Being able to preserve biological samples is critical, especially for research involving multi-site studies and collaborations, where samples are often collected at different times and locations. The viability and integrity of preserved samples are of paramount importance while preserving biological 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.



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

Specificity	Clone	Specificity	Clone
CD11c	B-Ly6	CD197 (CCR7)	2-L1-A
CD14	MPHIP9	CD186 (CXCR6)	13B 1E5
CD185 (CXCR5)	RF8B2	CD127	HIL-7R-M21
CD19	SJ25C1	CD134	ACT35
CD25	2A3	CD28	L293
CD27	M-T271	CD272	J168-540
CD278	DX29	CD8	SK1
CD279	EH12.1	HLA-DR	G46-6
CD3	UCHT1	CD16	3G8
CD357 (GITR)	V27-580	CD183	1C6/CXCR3
CD366 (Tim3)	7D3	CD196 (CCR6)	11A9
CD4	SK3	CD137	4B4-1
CD45RA	HI100	CD161	HP-3G10
CD56	NCAM16.2	IgM	G20-127
CD62L	DREG-56	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	CD8α	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.

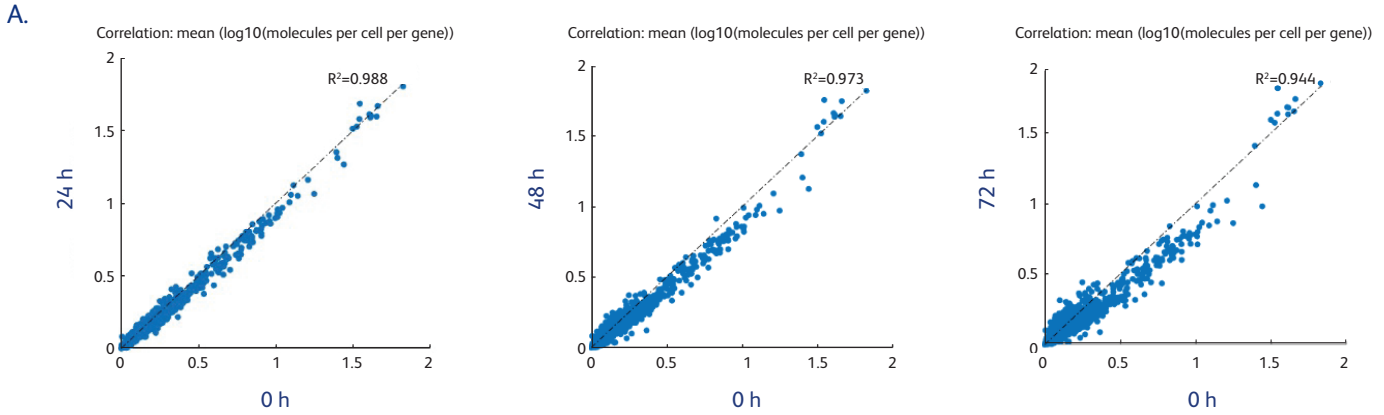
Time point	Human PBMC viability (%)	
	Donor 1	Donor 2
0 h	96.25	95.21
24 h	83.17	86.81
48 h	79.18	78.35
72 h	77.63	76

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

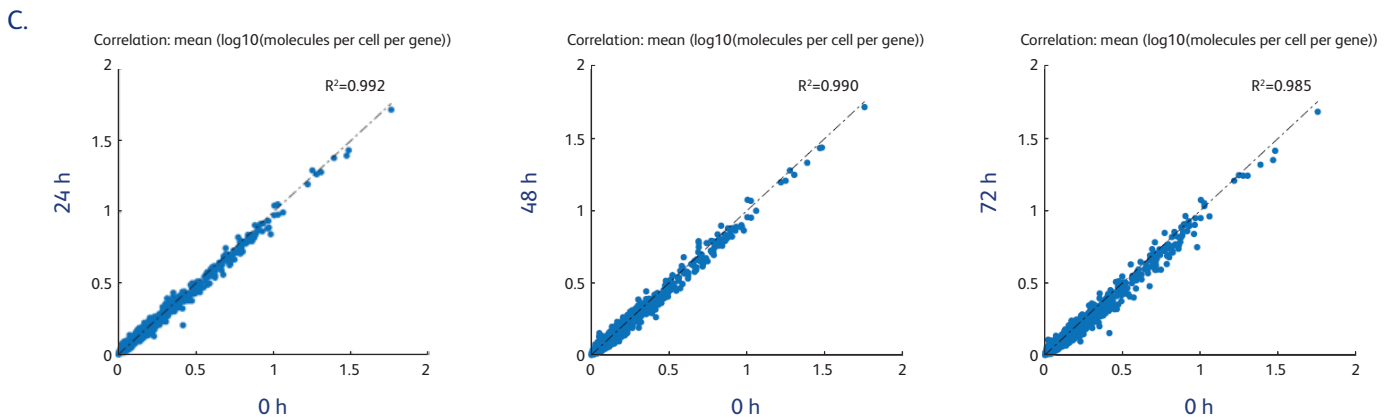
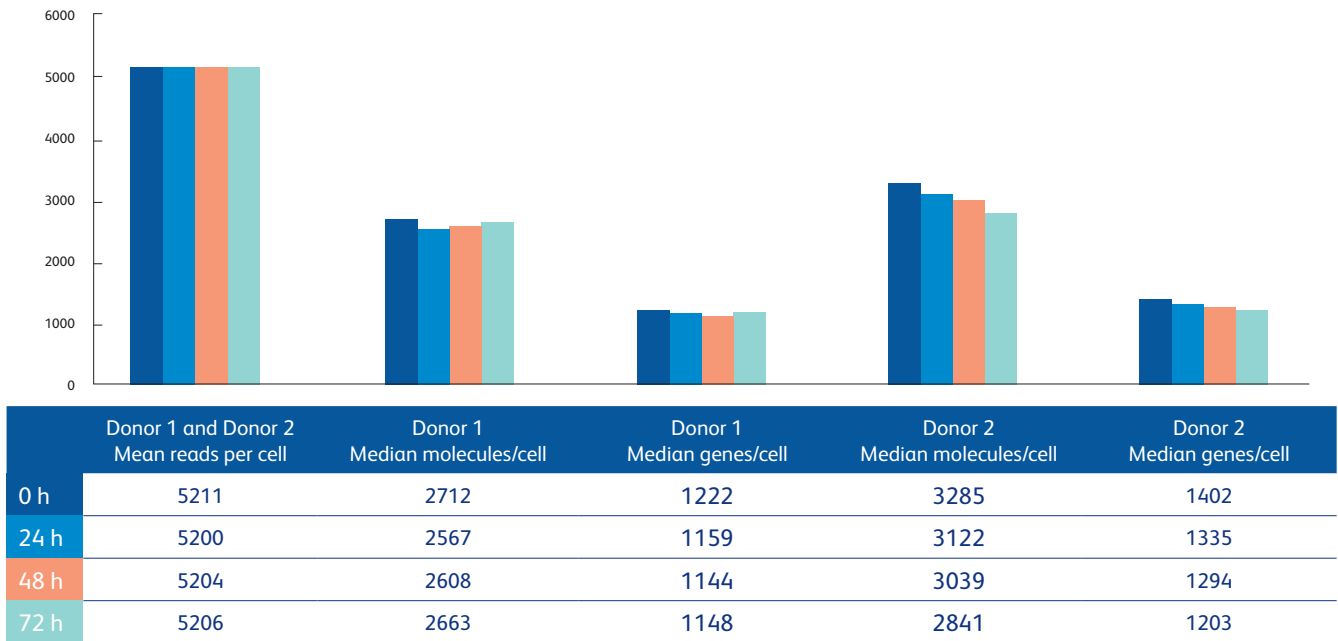
Human PBMCs, both controls and BD® OMICS-Guard Buffer-preserved samples were stained with DRAQ7™ and Calcein AM and the cell viability metric was quantified with the BD Rhapsody™ Scanner.

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

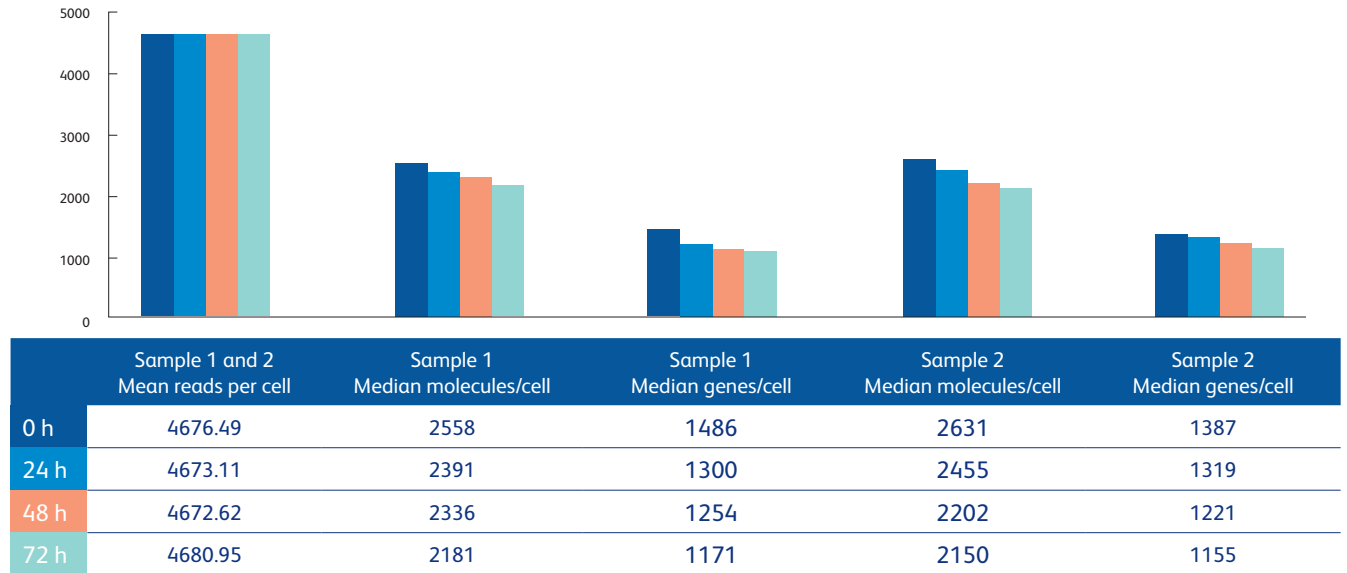
BD® OMICS-Guard Sample Preservation Buffer preserves cellular transcriptomic integrity and expression profiles for up to 72 hours at 4 °C. In this CITE-seq study, gene expression of single cells was profiled using the BD Rhapsody™ WTA Assay. Results showed high concordance of single-cell gene expression 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.



### B. Human PBMC WTA assay sensitivity



## D. Mouse splenocyte WTA assay sensitivity



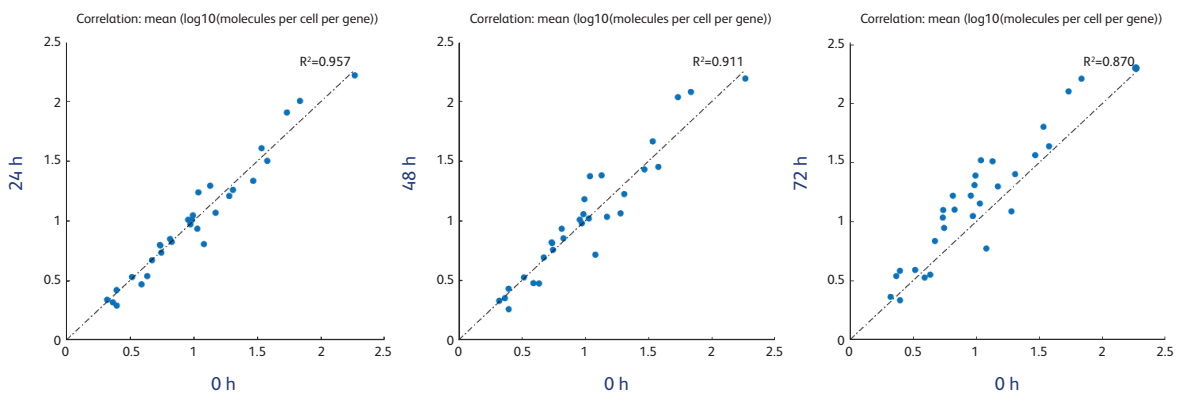
**Figure 3. Gene expression and WTA sensitivity are in high concordance over the 72-h storage period.**

**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<sup>™</sup> 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<sup>®</sup> OMICS-Guard Buffer preserves proteome profiles in single-cell and tissue samples

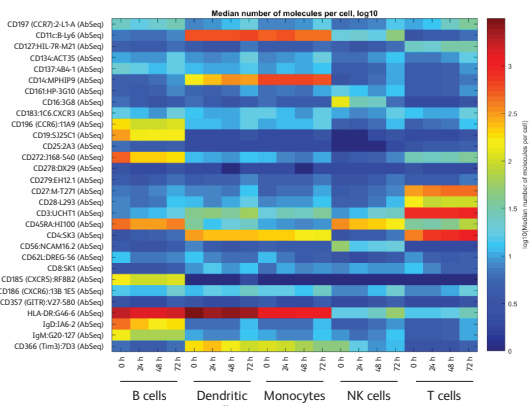
BD<sup>®</sup> OMICS-Guard Sample Preservation Buffer preserves proteomic integrity and expression profiles for up to 72 hours at 4 °C. Single-cell surface protein expression was quantified using a 30-plex BD<sup>®</sup> AbSeq Ab-Oligo Panel. High protein expression correlation and comparable AbSeq sensitivity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

**A.**

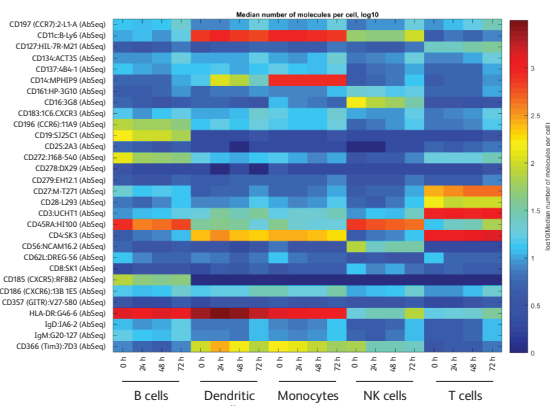


B.

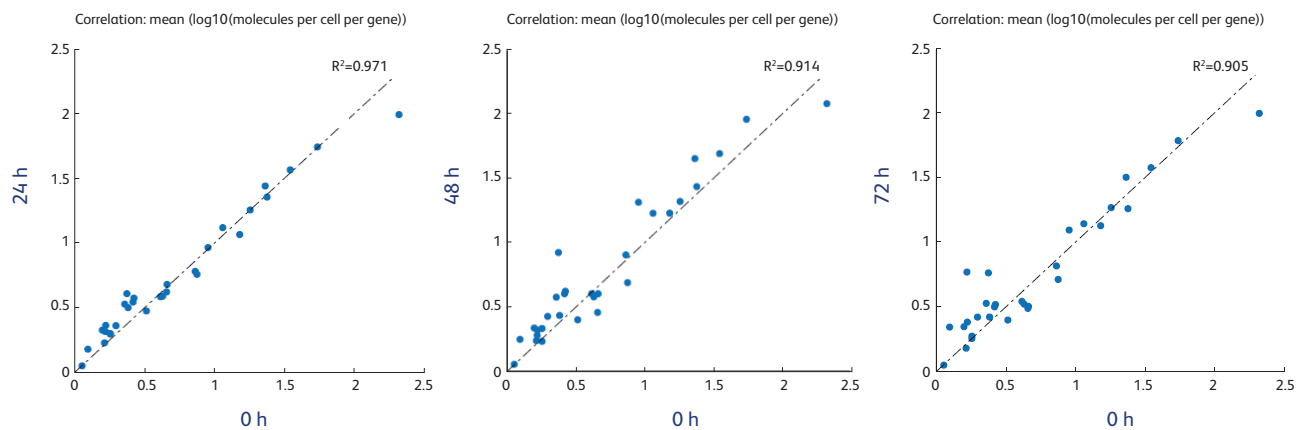
Human PBMC donor 1



Human PBMC donor 2

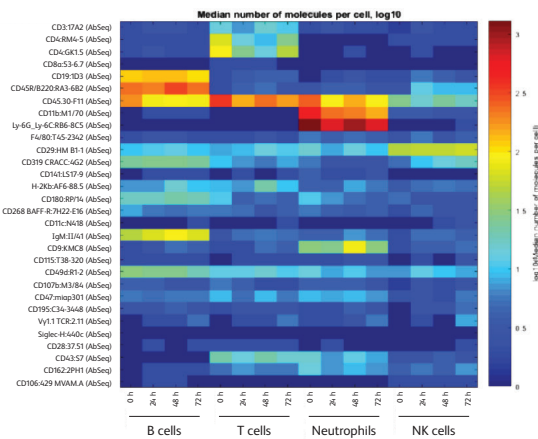


C.



D.

Mouse spleen tissue sample 1



Mouse spleen tissue sample 2

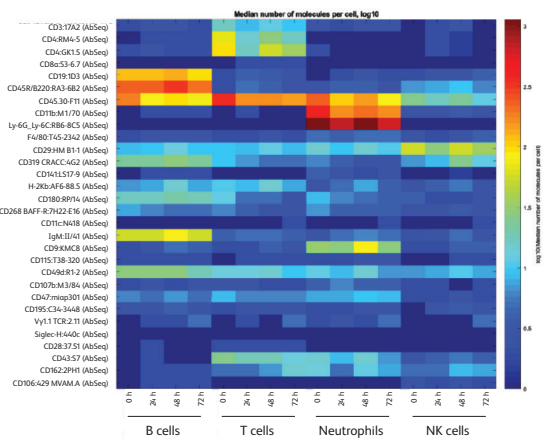
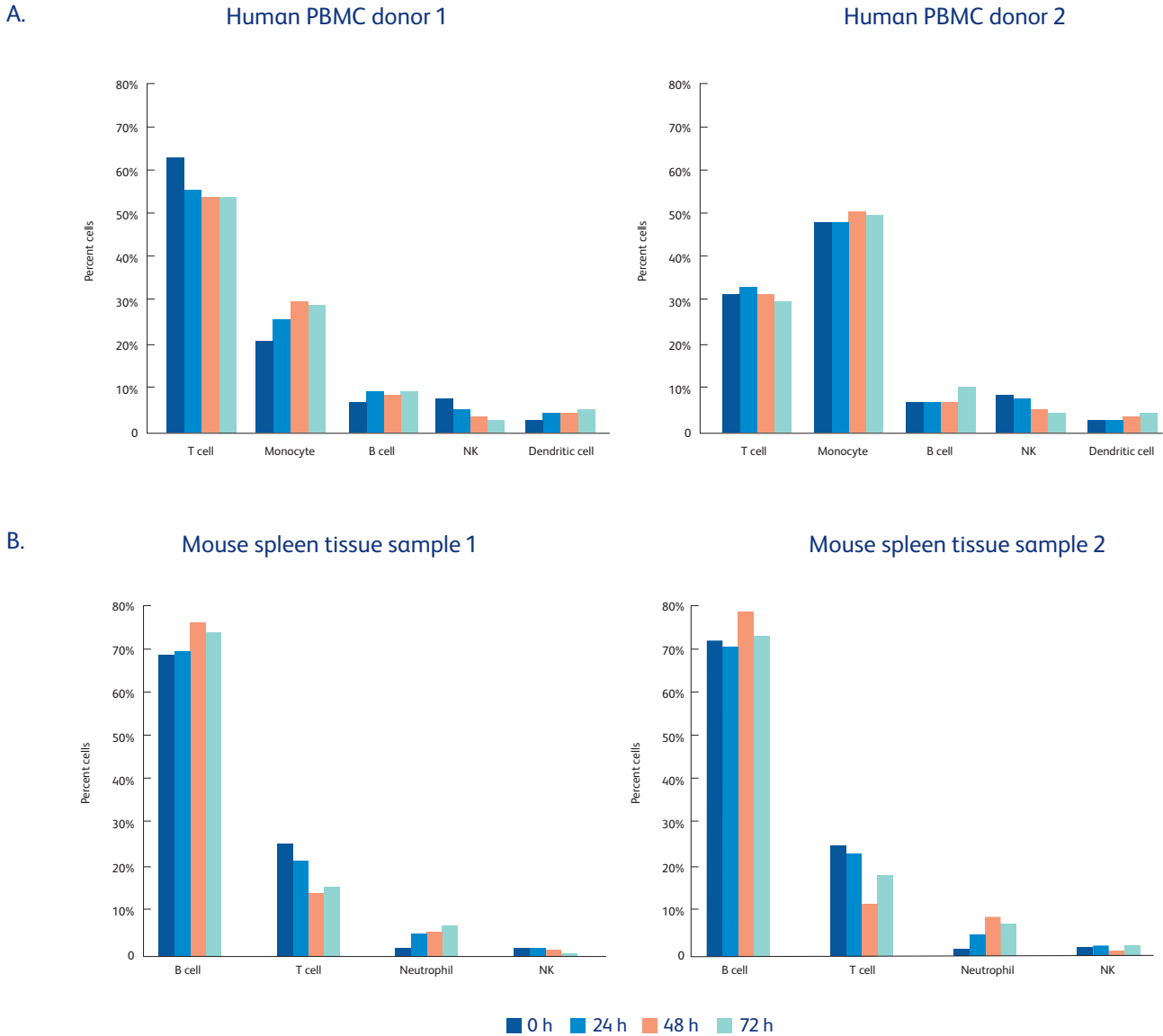


Figure 4. AbSeq expression and sensitivity are in high concordance over the 72-h storage period.

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<sup>2</sup>>0.8) and mouse splenocytes (4C: sample 1, R<sup>2</sup>>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<sup>®</sup> 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<sup>®</sup> OMICS-Guard Buffer-preserved samples using flow cytometry analyses

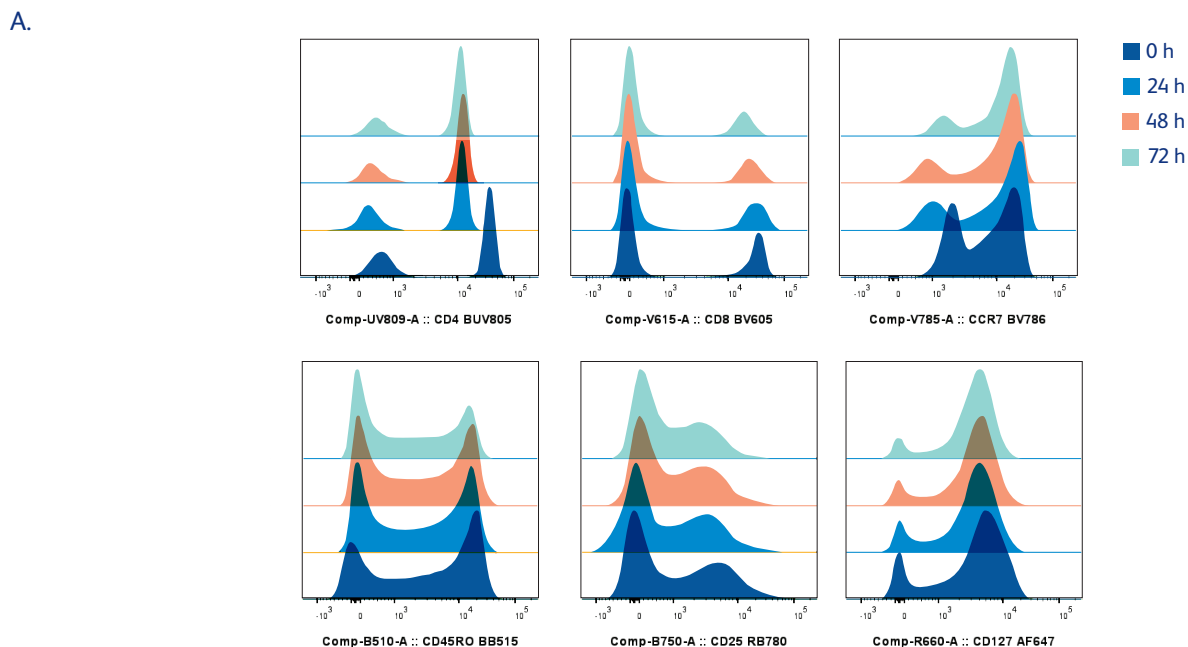
Human PBMC samples and mouse spleen tissues from the aforementioned CITE-seq experiments (Figure 1A) were analyzed by flow cytometry for surface protein expression profiling.

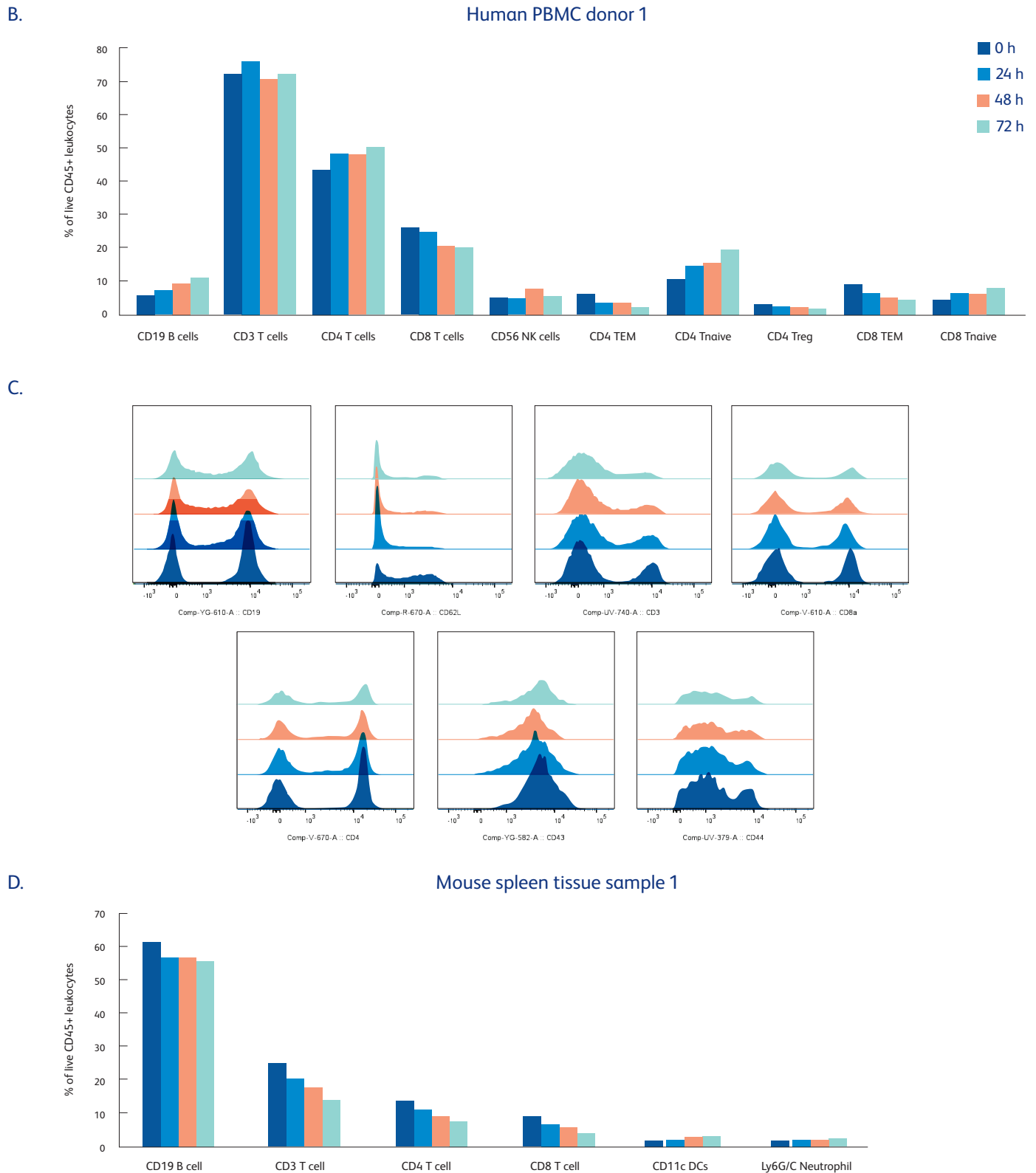
Human 13-plex flow panel			Mouse 11-plex flow panel		
Specificity	Dye	Clone	Specificity	Dye	Clone
CD45	BUV395	HI30	CD3	BUV737	17A2
CD56	BUV615	NCAM16.2	CD4	BV650	GK1.5
CD4	BUV805	SK3	CD8a	BV605	53-6.7
CD19	BV421	HIB19	CD19	PE-CF594	1D3
CD3	BV480	UCHT1	CD11c	BV421	HL3
CD8	BV605	SK1	CD45	PE-Cy7	30-F11
CD16	BV650	3G8	Ly6-G/C	R718	RB6-8C5
CCR7	BV786	2-L1-A	CD44	BUV395	IM7
CD45RO	BB515	UCHL1	CD43	PE	S7
CD14	PE-CF594	MφP9	CD62L	APC	MEL-14
CD25	RB780	2A3	CD49b	FITC	DX5
CD127	Alexa Fluor™ 647	HIL-7R-M21			
FVS780 (APC-Cy7 channel)					

**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<sup>®</sup> 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<sup>®</sup> OMICS-Guard Buffer can preserve surface protein epitopes for single-cell proteomics analyses and protect the cell composition of single-cell or tissue samples.



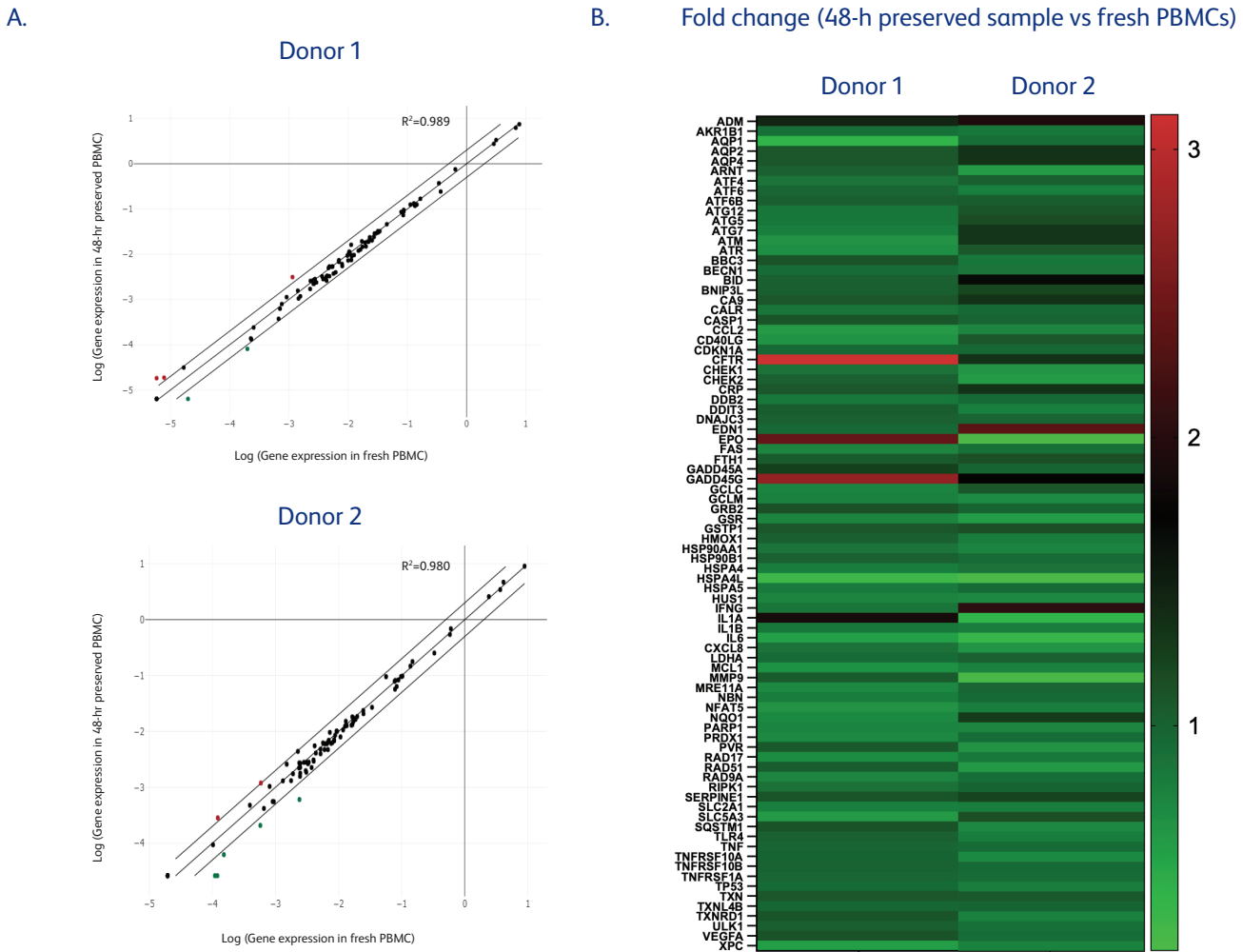


**Figure 7. Proteomic profiles and major immune cell population frequencies of samples revealed by flow cytometry.**

**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.



**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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