# Protect your samples, guard your science

BD® OMICS-Guard Sample Preservation Buffer



#### Biological sample preservation is a critical need







#### Collaborations

When samples need to be processed in a centralized location

#### Clinical samples for research

When it's hard to predict when samples can be collected and/or processed

#### Large-scale studies

When there are too many samples to process at the same time

### Introducing BD® OMICS-Guard Sample Preservation Buffer

A simple solution for biological sample preservation to provide flexibility when samples cannot be processed at the same time or need to be transported between study sites.



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: PBMC and tissue samples



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



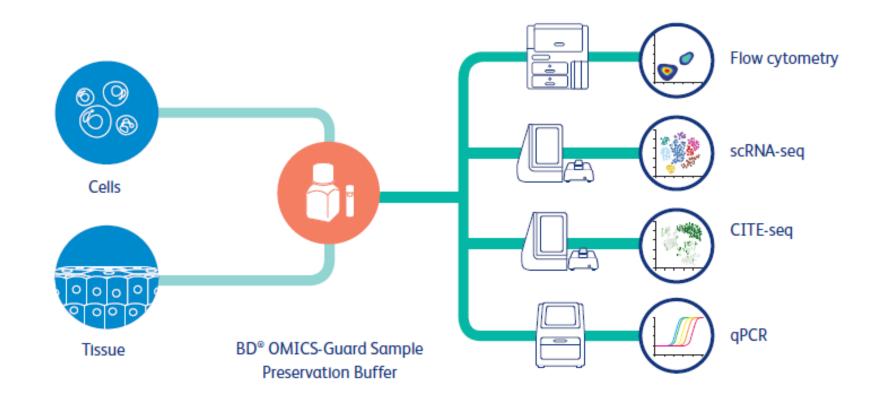
PFA-free reagent with lower health risks



#### One-step sample preservation workflow

	Single-cell suspension	Tissue
Recommended usage	1 to 10 million cells per 1 mL BD <sup>®</sup> OMICS-Guard Preservation Buffer	30 to 50 mg of tissue per 20 mL BD <sup>®</sup> OMICS-Guard Preservation Buffer
Storage temperature	4	°C
Storage duration	Up to 7	<sup>7</sup> 2 hours
Sample preservation protocol	<ol> <li>Collect single cells/dissociated single cells in suspension and spin at 400 x g for 5 minutes.</li> <li>Discard supernatant and resuspend the cells in the BD® OMICS-Guard Buffer as recommended above.</li> <li>Note: Handle the buffer-containing tubes/bottle under aseptic conditions. Cells can be stored in Eppendorf tubes or equivalent.</li> <li>Place cells at 4 °C for up to 72 hours.</li> <li>After storage, spin cells at 800 x g for 5 minutes and discard the supernatant to remove the BD® OMICS- Guard Buffer. No further washing is required.</li> <li>Resuspend the cells in desired buffer for downstream applications.</li> </ol>	<ol> <li>Section tissues into pieces and immediately place them into the BD® OMICS-Guard Buffer.</li> <li>Note: Handle the buffer-containing tubes/bottle under aseptic conditions.</li> <li>Place the preserved tissue at 4 °C for up to 72 hours.</li> <li>After storage, dissociate the tissue by the desired method for single-cell application, spin the cells at 800 x g for 5 minutes and discard supernatant to remove the BD® OMICS-Guard Buffer. No further washing is required.</li> <li>Resuspend the cells in desired buffer for downstream applications.</li> </ol>

BD<sup>®</sup> OMICS-Guard Buffer-preserved samples can be used in a variety of downstream research applications

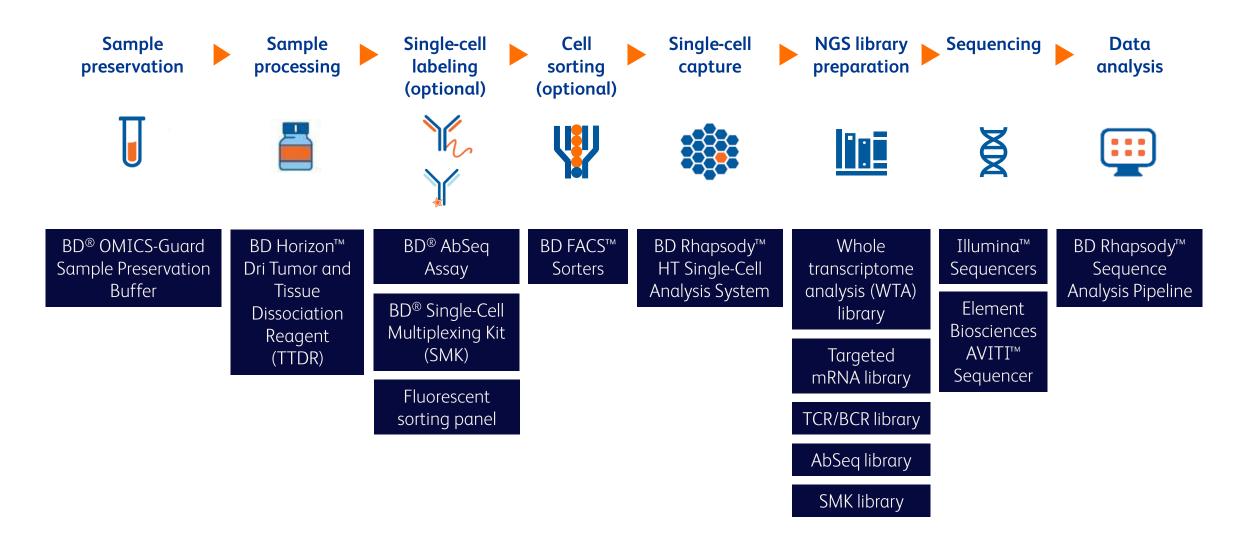


For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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



#### Single source for single-cell multiomics research



### Improve Ab-oligo signal with the BD® AbSeq Enhancer Kit for BD® OMICS-Guard Buffer-preserved samples in CITE-seq

Preserve your samples for scRNA-seq experiments

BD® OMICS-Guard Sample Preservation Buffer Bottle (50 mL) or 12 vials/kit, 1 test/vial (1 mL)

Available in **two formats:** 50-mL bottle to accommodate tissue preservation, where larger volumes may be required, or 1-mL single-use vials for single-cell experiments Preserve your samples for CITE-seq experiments

BD® OMICS-Guard Sample Preservation Buffer Bottle (50 mL) or 12 vials/kit, 1 test/vial (1 mL)

+

BD<sup>®</sup> AbSeq Enhancer Kit

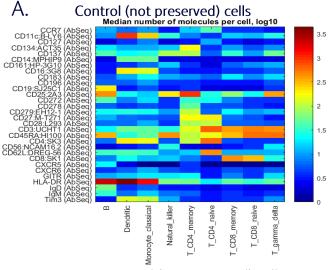
BD® AbSeq Enhancer 1, 50 µL

BD® AbSeq Enhancer 2, 50 µL

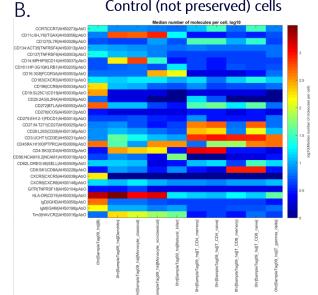
BD® AbSeq Enhancer 3, 50 µL

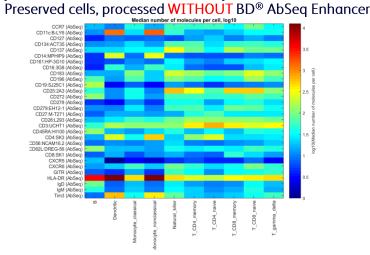
Use the BD<sup>®</sup> AbSeq Enhancer Kit for high-quality AbSeq data

#### Improve Ab-oligo signal with the BD<sup>®</sup> AbSeq Enhancer Kit for BD<sup>®</sup> OMICS-Guard Buffer-preserved samples in CITE-seq

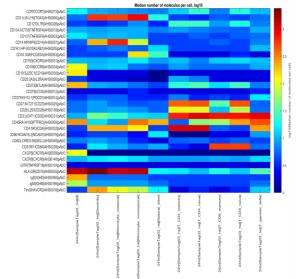


Control (not preserved) cells





Preserved cells, processed WITH the BD® AbSeg Enhancer Kit



Heatmaps of AbSeq performance with and without the addition of the BD® AbSeq Enhancer Kit. Heat maps of AbSeq median molecules per cell expression for different cell types are shown when the BD® AbSeq Enhancer Kit is either not added to the protocol (A) or added to the protocol (B) when using preserved cells. A high amount of nonspecific background signal can be observed in (A) when no enhancer is used as compared to (B). In (B), AbSeq performance closely matches that of the control data (left plot).

#### 😂 BD

# Improve Ab-oligo signal with the BD® AbSeq Enhancer Kit for BD® OMICS-Guard Buffer-preserved samples in CITE-seq

BD<sup>®</sup> OMICS-Guard Buffer allows antibody staining before or after sample preservation. For CITE-seq applications using BD<sup>®</sup> AbSeq Ab-Oligos **AFTER** sample preservation, use of the BD<sup>®</sup> AbSeq Enhancer Kit (Cat. no. 570750) is highly recommended. BD<sup>®</sup> AbSeq Enhancers can be added to the BD Fc Block<sup>M</sup> Reagent step or used separately prior to single-cell staining with BD<sup>®</sup> AbSeq Ab-Oligos.

Note: Staining with BD<sup>®</sup> AbSeq Ab-Oligos BEFORE sample preservation or staining with the BD<sup>®</sup> Single-Cell Multiplexing Kit ONLY after sample preservation does not require the use of the BD<sup>®</sup> AbSeq Enhancer Kit.

#### Staining protocol using human PBMC

1. Prepare the Human BD Fc Block™ Reagent as follows:

Component	Volume/sample (µL)	Volume/sample with overage (µL)
Stain buffer	65	78
Human BD Fc Block™ Reagent	5	6
Total	70	84

- To the 70 µL Human BD Fc Block™ Reagent mixture, add 10 µL of each of the three BD<sup>®</sup> AbSeq Enhancers for a total of 30 µL.
   Note: The Human BD Fc Block™ Reagent and BD<sup>®</sup> AbSeq Enhancers mixture should have a final volume of 100 µL.
- 3. Spin the cell suspension from the preserved sample at 800 x g and remove the supernatant without disturbing the pellet.
- 4. Add the 100  $\mu$ L final mix and resuspend the cell pellet.
- 5. Incubate the cells at room temperature for 10 minutes.
- 6. Add BD® AbSeq Ab-Oligo cocktails per the standard AbSeq staining protocols.\*

#### For BD Rhapsody™ Single-Cell Analysis System users

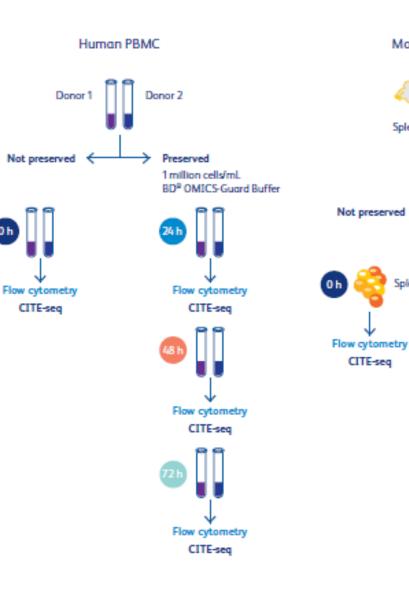
For cells/tissues preserved in BD<sup>®</sup> OMICS-Guard Buffer, extend the lysis time in the single-cell capture workflow from 2 minutes to 5 minutes for optimal results.

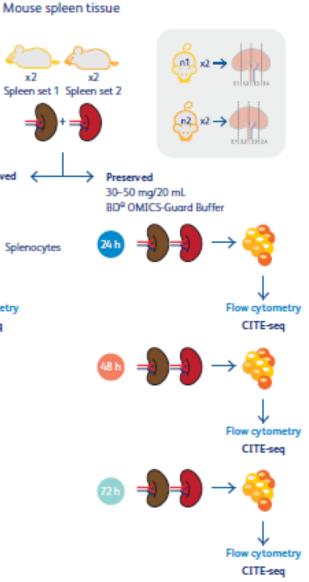
BD
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\*100-µL 2X BD<sup>®</sup> AbSeq Ab-Oligo labeling MasterMix, incubate 30–60 minutes on ice. See BD Rhapsody<sup>™</sup> System Single-Cell Labeling with BD<sup>®</sup> AbSeq Ab-Oligos (1 plex to 40 plex) Protocol 10 (Doc ID: 23-24262).

# Overview of experimental design

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.





#### 30-plex BD<sup>®</sup> AbSeq Panels used in the study

#### BD® AbSeq Immune Discovery Panel used for human PBMC CITE-seq 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 Panel used for mouse spleen CITE-seq analyses

940135IgMII/41940179CD49dR1-2940198CD115T38-320940186CD106429 MVAM.A940120CD2837.51940200CD9KMC8940321CD11cN418940334Vγ1.1 TCR2.11940108CD4RM4-5940345CD8a53-6.7940111CD191D3940008CD11bM1/70940414CD141LS17-9940300CD268 BAFF-R7H22-E16940410Siglec-H440c940498CD195C34-3448940483CD107bM3/84940446CD47miap301940145CD43S7940184CD1622PH1940164H-2KbAF6-88.5940410CD45R/B220RA3-6B2940333CD317A2940471CD4GK1.5940459CD180RP/14		c :::::	
940179         CD49d         R1-2           940198         CD115         T38-320           940186         CD106         429 MVAM.A           940120         CD28         37.51           940200         CD9         KMC8           940321         CD11c         N418           940334         Vy1.1TCR         2.11           940108         CD4         RM4-5           940345         CD8a         53-6.7           940111         CD19         1D3           940008         CD141         LS17-9           940131         F4/80         T45-2342           940330         CD268 BAFF-R         7H22-E16           940410         Siglec-H         440c           940483         CD107b         M3/84           940483         CD107b         M3/84           940483         CD107b         M3/84           940145         CD43         S7           940184         CD162         2PH1           940164         H-2Kb         AF6-88.5           940110         CD45R/B220         RA3-6B2           940110         CD45R/B220         RA3-6B2           940110         CD45R/B220 </th <th>Cat. no.</th> <th>Specificity</th> <th>Clone</th>	Cat. no.	Specificity	Clone
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940186CD106429 MVAM.A940120CD2837.51940200CD9KMC8940321CD11cN418940334Vγ1.1TCR2.11940108CD4RM4-5940345CD8a53-6.7940111CD191D3940008CD11bM1/70940414CD141LS17-9940131F4/80T45-2342940300CD268 BAFF-R7H22-E16940410Siglec-H440c940498CD195C34-3448940446CD47miap301940145CD43S7940184CD1622PH1940164H-2KbAF6-88.5940410CD45R/B220RA3-6B2940110CD45R/B220RA3-6B2940110CD45R/B220RA3-6B2940110CD45R/B220RA3-6B2940471CD4GK1.5940459CD180RP/14	940179	CD49d	R1-2
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940334Vγ1.1 TCR2.11940108CD4RM4-5940345CD8α53-6.7940111CD191D3940008CD11bM1/70940414CD141LS17-9940131F4/80T45-2342940330CD268 BAFF-R7H22-E16940410Siglec-H440c940483CD195C34-3448940483CD107bM3/84940145CD43S7940184CD1622PH1940164H-2KbAF6-88.5940410CD29HM B1-1940158CD29HM B1-1940110CD45R/B220RA3-6B2940119Ly6G & Ly-6CRB6-8C5940471CD4GK1.5940459CD180RP/14	940200	CD9	KMC8
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940414CD141LS17-9940131F4/80T45-2342940330CD268 BAFF-R7H22-E16940410Siglec-H440c940498CD195C34-3448940483CD107bM3/84940446CD47miap301940145CD43S7940184CD1622PH1940164H-2KbAF6-88.5940411CD319 CRACC4G2940158CD29HM B1-1940110CD45R/B220RA3-6B2940119Ly6G & Ly-6CRB6-8C5940471CD4GK1.5940459CD180RP/14	940111	CD19	1D3
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940330         CD268 BAFF-R         7H22-E16           940410         Siglec-H         440c           940498         CD195         C34-3448           940483         CD107b         M3/84           940446         CD47         miap301           940145         CD43         S7           940184         CD162         2PH1           940164         H-2Kb         AF6-88.5           940411         CD319 CRACC         4G2           940158         CD29         HM B1-1           940110         CD45R/B220         RA3-6B2           940119         Ly6G & Ly-6C         RB6-8C5           940471         CD4         GK1.5           940459         CD180         RP/14	940414	CD141	LS17-9
940410Siglec-H440c940498CD195C34-3448940483CD107bM3/84940446CD47miap301940145CD43S7940184CD1622PH1940164H-2KbAF6-88.5940411CD319 CRACC4G2940158CD29HM B1-1940110CD45R/B220RA3-6B2940119Ly6G & Ly-6CRB6-8C5940471CD4GK1.5940459CD180RP/14	940131	F4/80	T45-2342
940498         CD195         C34-3448           940483         CD107b         M3/84           940446         CD47         miap301           940145         CD43         S7           940184         CD162         2PH1           940164         H-2Kb         AF6-88.5           940158         CD29         HM B1-1           940110         CD45R/B220         RA3-6B2           940119         Ly6G & Ly-6C         RB6-8C5           940471         CD4         GK1.5           940459         CD180         RP/14	940330	CD268 BAFF-R	7H22-E16
940483         CD107b         M3/84           940446         CD47         miap301           940145         CD43         S7           940184         CD162         2PH1           940164         H-2Kb         AF6-88.5           94011         CD319 CRACC         4G2           940158         CD29         HM B1-1           940110         CD45R/B220         RA3-6B2           940119         Ly6G & Ly-6C         RB6-8C5           940471         CD4         GK1.5           940459         CD180         RP/14	940410	Siglec-H	440c
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940145         CD43         S7           940184         CD162         2PH1           940164         H-2Kb         AF6-88.5           94011         CD319 CRACC         4G2           940158         CD29         HM B1-1           940110         CD45R/B220         RA3-6B2           940119         Ly6G & Ly-6C         RB6-8C5           940471         CD4         GK1.5           940459         CD180         RP/14	940483	CD107b	M3/84
940184         CD162         2PH1           940164         H-2Kb         AF6-88.5           940411         CD319 CRACC         4G2           940158         CD29         HM B1-1           940110         CD45R/B220         RA3-6B2           940119         Ly6G & Ly-6C         RB6-8C5           940433         CD3         17A2           940459         CD180         RP/14	940446	CD47	miap301
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940411         CD319 CRACC         4G2           940158         CD29         HM B1-1           940110         CD45R/B220         RA3-6B2           940119         Ly6G & Ly-6C         RB6-8C5           940333         CD3         17A2           940471         CD4         GK1.5           940459         CD180         RP/14	940184	CD162	2PH1
940158         CD29         HM B1-1           940110         CD45R/B220         RA3-6B2           940119         Ly6G & Ly-6C         RB6-8C5           940333         CD3         17A2           940471         CD4         GK1.5           940459         CD180         RP/14	940164	H-2Kb	AF6-88.5
940110         CD45R/B220         RA3-6B2           940119         Ly6G & Ly-6C         RB6-8C5           940333         CD3         17A2           940471         CD4         GK1.5           940459         CD180         RP/14	940411	CD319 CRACC	4G2
940119         Ly6G & Ly-6C         RB6-8C5           940333         CD3         17A2           940471         CD4         GK1.5           940459         CD180         RP/14	940158	CD29	HM B1-1
940333         CD3         17A2           940471         CD4         GK1.5           940459         CD180         RP/14	940110	CD45R/B220	RA3-6B2
940471         CD4         GK1.5           940459         CD180         RP/14	940119	Ly6G & Ly-6C	RB6-8C5
940459 CD180 RP/14	940333	CD3	17A2
	940471	CD4	GK1.5
9/i0320 CD/i5 30_E11	940459	CD180	RP/14
	940320	CD45	30-F11

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### Cell capture and cell calling metrics

#### A. Human

Sample	Cells to capture	Number of retrieved beads with a viable cell	Cell retention rate	Cell subsample	Putative cells
0 h	20,000	14,315	88.50%	4,000	3,644
24 h	30,000	19,113	89.20%	4,000	2,939
48 h	30,000	16,540	89.40%	4,000	2,668
72 h	30,000	16,363	89.40%	4,000	2,716

#### B. Mouse

Sample	Cells to capture	Number of retrieved beads with a viable cell	Cell retention rate	Cell subsample	Putative cells
0 h	40,000	25,043	98.10%	4,000	3,786
24 h	40,000	27,278	97.10%	4,000	3,822
48 h	40,000	23,102	97.00%	4,000	4,319
72 h	40,000	21,322	87.50%	4,000	4,142

Cell capture and putative cell calling. 20–30K human PBMCs (A) and 40K splenocytes from mouse spleen (B) were loaded into the BD Rhapsody<sup>™</sup> System. The number of retrieved beads with a viable cell value is shown in the table as reported from the BD Rhapsody<sup>™</sup> Scanner. For both human PBMCs and mouse splenocytes, beads were subsampled to 4,000. Putative cells is also reported in the table and was determined based on mRNA cell calling in the BD Rhapsody<sup>™</sup> Sequence Analysis Pipeline.

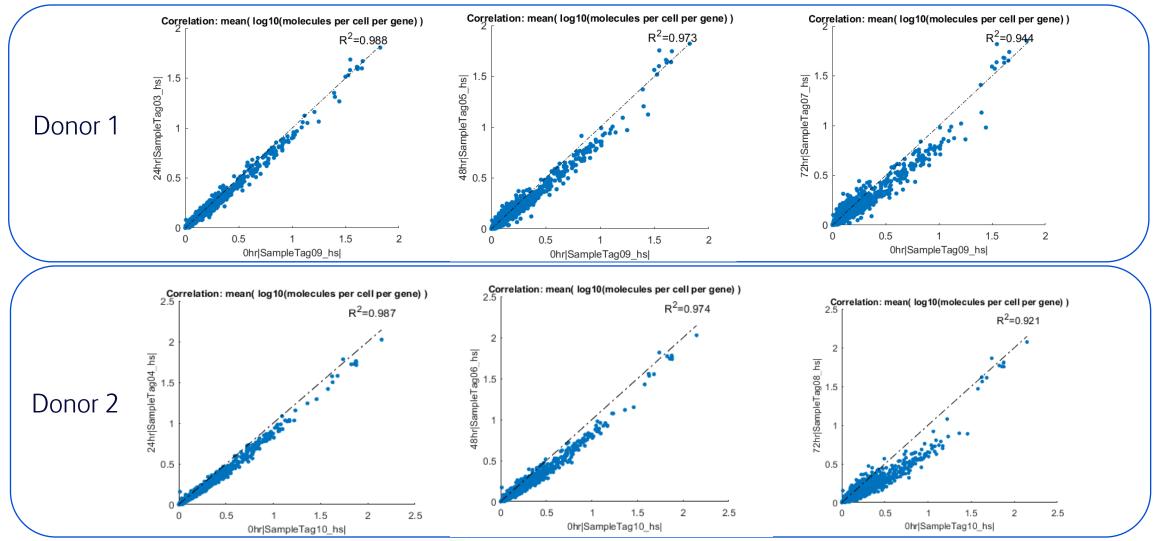


## BD<sup>®</sup> OMICS-Guard Buffer preserves cell viability in human single-cell and mouse tissue samples

Time point	Human PBMC viability (%): Donor 1	Human PBMC viability (%): Donor 2	Mouse spleen viability(%)
0 h	96.25	95.21	92.1
24 h	83.17	86.81	72.5
48 h	79.18	78.35	80.0
72 h	77.63	76	84.3

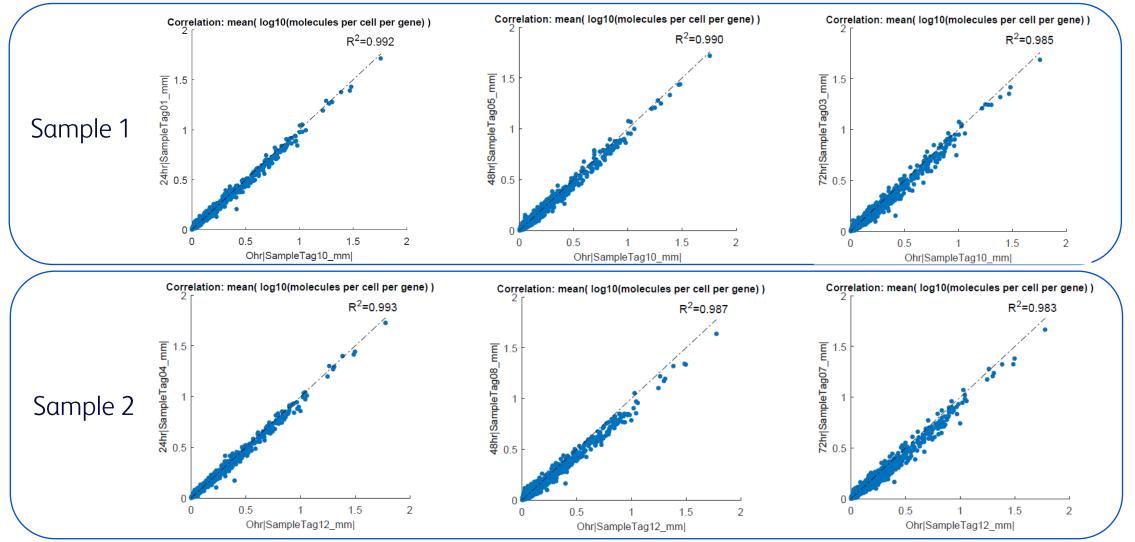
Cell viability of hPBMCs and mouse splenocytes. For hPBMC—PBMCs from two different donors were co-stained with BD® AbSeq Ab-Oligos and BD® SMK, followed by staining with DRAQ7<sup>™</sup> and Calcien AM at 37 °C for 5 minutes to obtain individual viability metrics using the BD Rhapsody<sup>™</sup> Scanner. A 1:1 ratio was calculated for pooling the different donors. For mouse spleen—following dissociation of bulk tissue and RBC lysis with BD Pharm Lyse<sup>™</sup> Solution, samples were simultaneous stained with mouse BD® AbSeq Ab-Oligos and BD® Single-Cell Multiplexing Set Rat Anti-Mouse MHC-H2 Class I (M1/42). Samples were then pooled and stained with DRAQ7<sup>™</sup> and Calcien AM at 37 °C for 5 minutes, and viability was quantified with the BD Rhapsody<sup>™</sup> Scanner.

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



Gene expression correlation between control human PBMC sample (0 h) vs preserved human PBMC samples at 24 h, 48 h and 72 h (top: Donor 1, bottom: Donor 2). R<sup>2</sup> correlation values were calculated using differentially expressed gene plots from WTA data on the BD Rhapsody<sup>™</sup> System. Gene expression is in high concordance over the 72-h storage period.

#### BD® OMICS-Guard Buffer preserves transcriptome and proteome profiles in mouse tissue samples

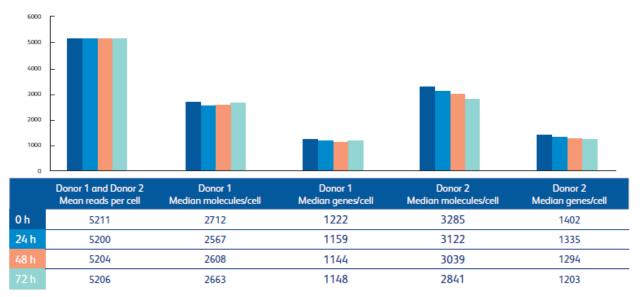


Gene expression correlation between control mouse spleen tissue (0 h) vs preserved mouse spleen tissues at 24 h, 48 h and 72 h (top: Sample 1, bottom: Sample 2).  $R^2$  correlation values were calculated using differentially expressed gene plots from WTA data on the BD Rhapsody<sup>TM</sup> System. Gene expression is in high concordance over the 72-h storage period.

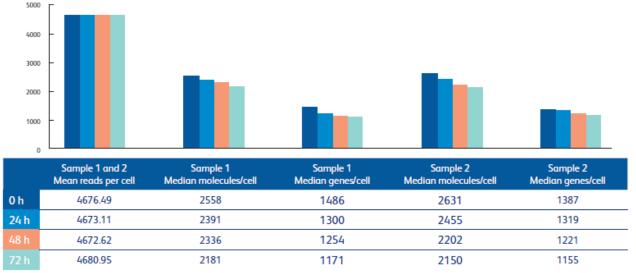
#### 🍪 BD

### Whole transcriptome analysis (WTA) assay sensitivity metrics

#### A. Human PBMC

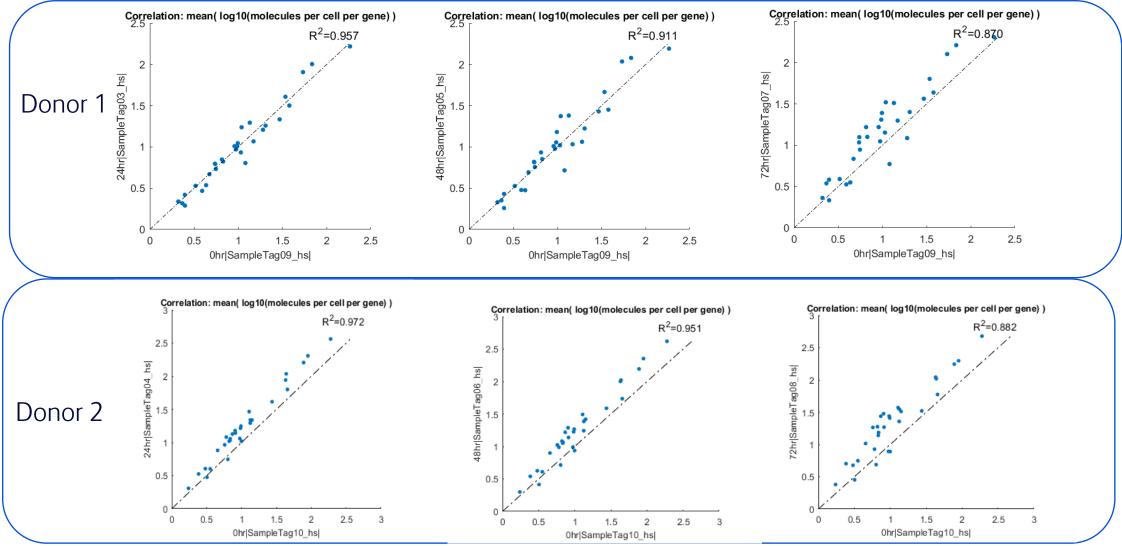


#### B. Mouse spleen tissue



WTA assay sensitivity represented by median molecules per cell (median transcripts per cell) and median genes per cell were compared among control samples (0 h) and preserved 24, 48, and 72-h human PBMCs (**A**) and mouse splenocytes (**B**). Sequencing data were normalized to the same read-depth and samples were demultiplexed.

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

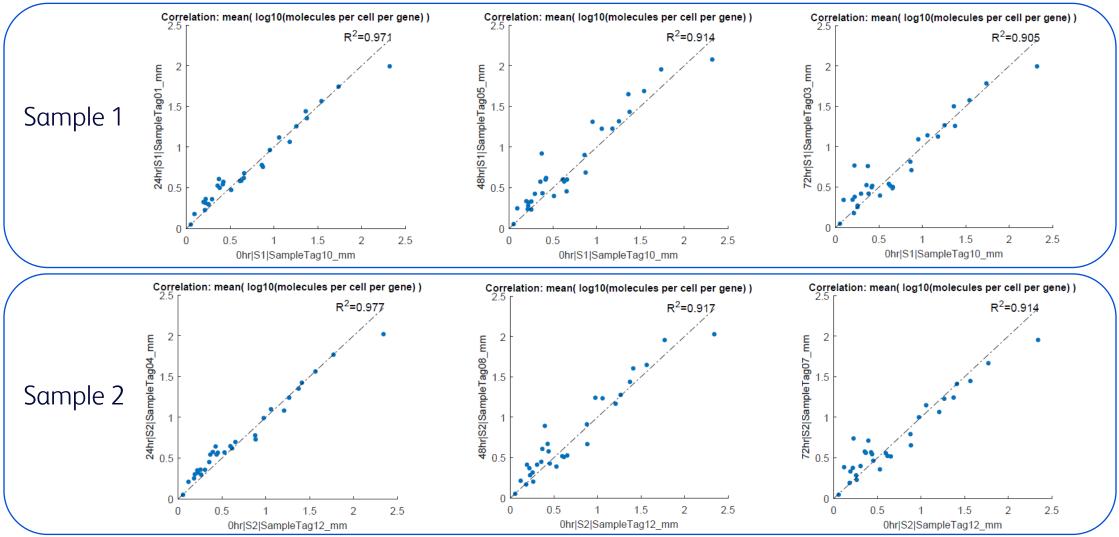


AbSeq expression representing protein expression correlation between control samples (0 h) and preserved samples at 24, 48 and 72-h time points in hPBMCs (top: Donor 1, bottom: Donor 2). Sequencing data were normalized to the same read-depth followed by demultiplexing of samples. AbSeq expression is in high concordance over the 72-h storage period.

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#### BD® OMICS-Guard Buffer preserves proteome profiles in mouse tissue samples



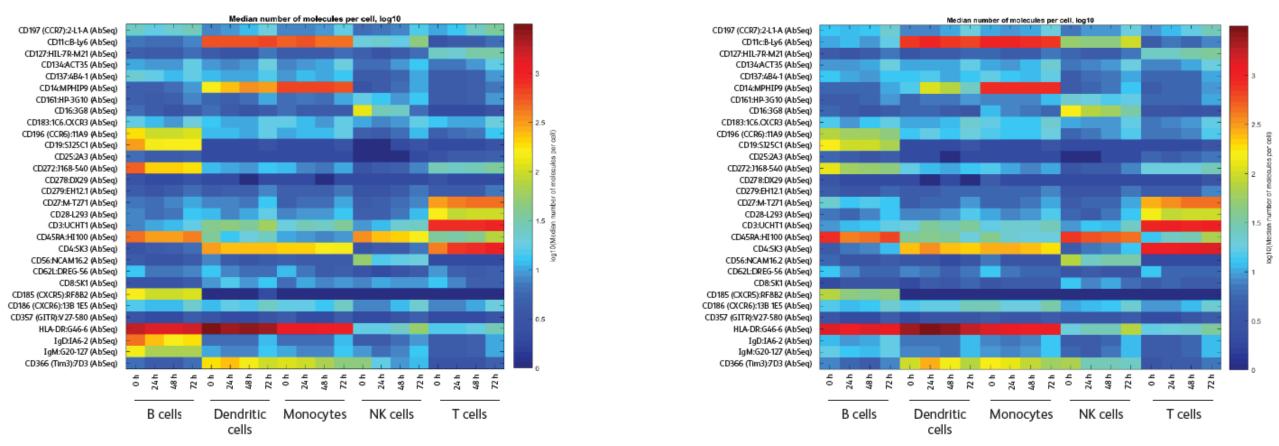
AbSeq expression representing protein expression correlation between control samples (0 h) and preserved samples at 24, 48 and 72-h time points in mouse splenocytes (top: Sample 1, bottom: Sample 2). Sequencing data were normalized to the same read-depth followed by demultiplexing of samples. AbSeq expression is in high concordance over the 72-h storage period.

#### BD

#### AbSeq sensitivity and specificity in human single-cell samples

Donor 1





AbSeq sensitivity represented by median molecules per cell of each BD<sup>®</sup> AbSeq Ab-Oligo used to stain human PBMCs in major PBMC cell types (B, T, NK and dendritic cells and monocytes). 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.

### AbSeq sensitivity and specificity in mouse tissue samples

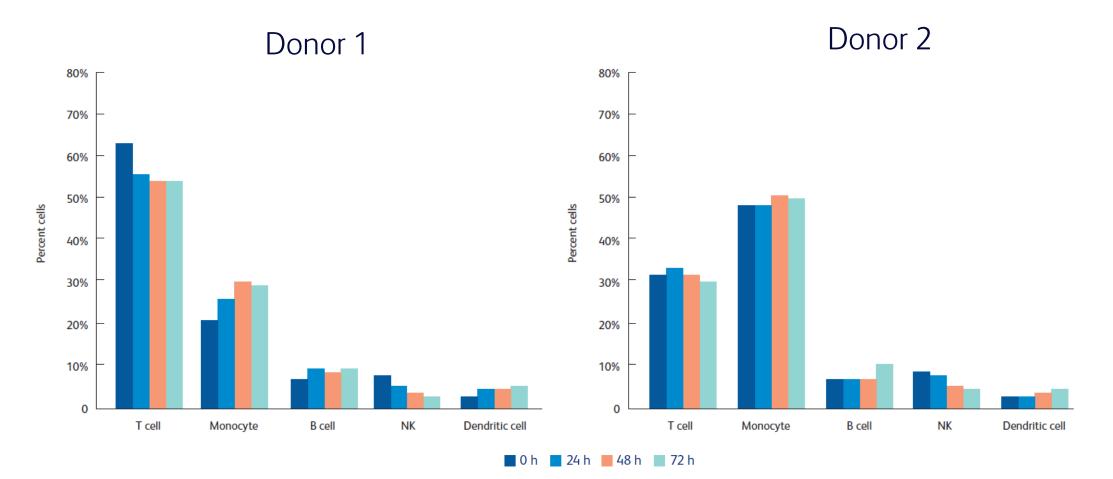
Median number of molecules per cell, log10 Median number of molecules per cell, log10 CD3:17A2 (AbSeq) CD3:17A2 (AbSeq) CD4:RM4-5 (AbSeq) CD4:RM4-5 (AbSeq) CD4:GK1.5 (AbSeq) CD4:GK1.5 (AbSeq) CD8a:53-6.7 (AbSeq) CD8a:53-6.7 (AbSea) CD19:1D3 (AbSeq) CD19:1D3 (AbSeq) CD45R/B220:RA3-6B2 (AbSeq) CD45R/B220:RA3-6B2 (AbSeq) 2.5 CD45.30-F11 (AbSeq) CD45.30-F11 (AbSeq) CD11b:M1/70 (AbSeq) CD11b:M1/70 (AbSeq) Ly-6G\_Ly-6C:RB6-8C5 (AbSeq) Ly-6G Ly-6C:RB6-8C5 (AbSeq) F4/80:T45-2342 (AbSeq) F4/80:T45-2342 (AbSeq) CD29:HM B1-1 (AbSeq) CD29:HM B1-1 (AbSeq) CD319 CRACC:4G2 (AbSeq) CD319 CRACC:4G2 (AbSeq) CD141:LS17-9 (AbSeq) CD141:LS17-9 (AbSeq) H-2Kb:AF6-88.5 (AbSeq) H-2Kb:AF6-88.5 (AbSeq) CD180:RP/14 (AbSeq) CD180:RP/14 (AbSeq) CD268 BAFF-R:7H22-E16 (AbSeq) 1.5 CD268 BAFF-R:7H22-E16 (AbSeq) CD11c:N418 (AbSeq) CD11c:N418 (AbSeq) IgM:II/41 (AbSeq) IgM:II/41 (AbSeq) CD9:KMC8 (AbSea) CD9:KMC8 (AbSeq) CD115:T38-320 (AbSeq) CD115:T38-320 (AbSeq) CD49d:R1-2 (AbSeq) CD49d:R1-2 (AbSeq) CD107b:M3/84 (AbSeq) CD107b:M3/84 (AbSeq) CD47:miap301 (AbSeq) CD47:miap301 (AbSea) CD195:C34-3448 (AbSeq) CD195:C34-3448 (AbSeq) Vy1.1 TCR:2.11 (AbSeq) Vy1.1 TCR:2.11 (AbSeq) 0.5 Siglec-H:440c (AbSeq) Siglec-H:440c (AbSeq) CD28:37.51 (AbSeq) CD28:37.51 (AbSeq) CD43:57 (AbSeq) CD43:57 (AbSea) CD162:2PH1 (AbSeq) CD162:2PH1 (AbSea) CD106:429 MVAMA (AbSeq) CD106:429 MVAM.A (AbSeq) 48 h 72h 48 h ő 48 h 6 ЧĦ 48 h 72h ÷ Чħ дh 48 h 72h 불 72h ÷ 24 h 18 h 72 h ч дh 48 h 72 h 5 Чh 72 h Чh 72 h ő B cells T cells Neutrophils NK cells T cells NK cells B cells Neutrophils

Sample 1

AbSeq sensitivity represented by median molecules per cell of each BD<sup>®</sup> AbSeq Ab-Oligo used to stain mouse splenocytes in major splenic cell types (B, T and NK cells and neutrophils). 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.

Sample 2

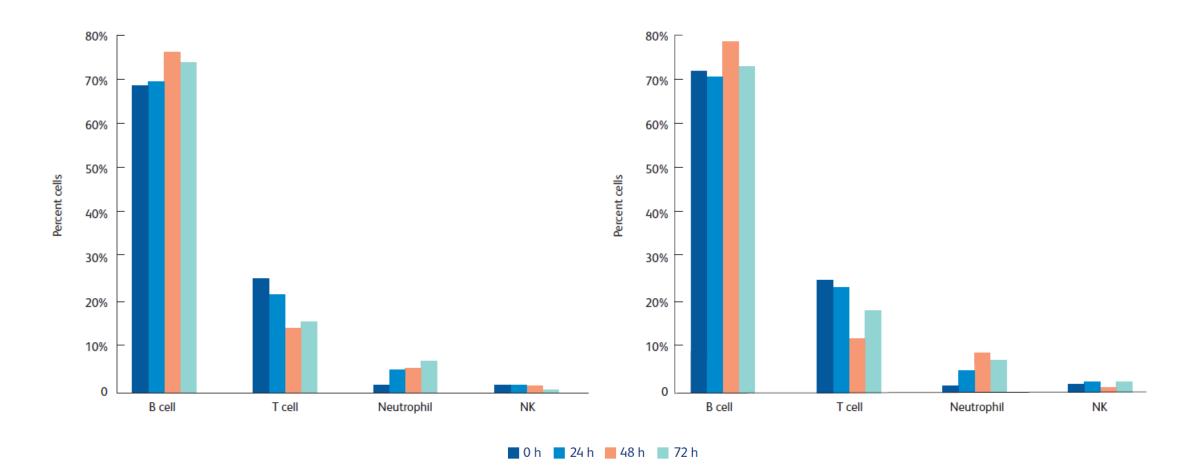
### Cell subpopulation frequencies of human single-cell samples are preserved by $\mathsf{BD}^{\circledast}$ OMICS-Guard Buffer



The frequencies of major cell populations in hPBMCs were maintained by BD<sup>®</sup> OMICS-Guard Buffer preservation across different time points (0, 24, 48, 72 h). Cell type annotation of hPBMCs was based on the immune cell type caller embedded in the BD Rhapsody<sup>™</sup> Sequence Analysis Pipeline.

#### 🍪 BD

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



The frequencies of major cell populations in mouse spleen tissues were maintained by BD<sup>®</sup> OMICS-Guard Buffer preservation across different time points (0, 24, 48, 72 h). Cell types in mouse spleen tissue were manually annotated based on gene expression.

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#### BD materials used in this study

Cat. no.	Product description	
570908	BD® OMICS-Guard Sample Preservation Buffer Kit	0 mar
570911	BD® OMICS-Guard Sample Preservation Buffer	
570750	BD® AbSeq Enhancer Kit	
666625	BD Rhapsody™ HT Xpress System Package	
633701	BD Rhapsody™ Scanner	Q BD Rhapsody
633773	BD Rhapsody™ cDNA Kit	CID https://www.withum
664887	BD Rhapsody™ Enhanced Cartridge Reagent Kit	
666262	BD Rhapsody™ 8-Lane Cartridge	-
633801	BD Rhapsody™ WTA Amplification Kit	
625970	BD® AbSeq Immune Discovery Panel	
Various	BD® AbSeq Ab-Oligos	
633781	BD® Human Single-Cell Multiplexing Kit	
626545	BD® Single-Cell Multiplexing Set Rat Anti-Mouse MHC-H2 Class I (M1/42)	Contraction of the second s
555899	BD Pharm Lyse™ Lysing Buffer	
564220	BD Pharmingen™ Human BD Fc Block™ Reagent	
553141	BD Pharmingen™ Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™ Reagent)	
554656	BD Pharmingen™ Stain Buffer (FBS)	



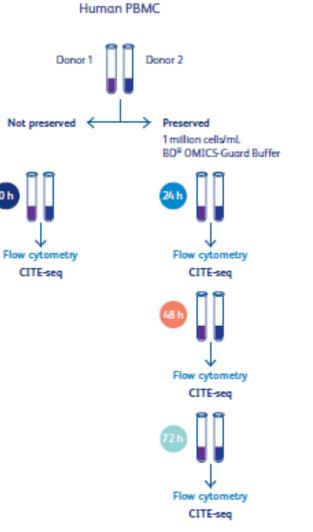
### Proteomic profiling of BD® OMICS-Guard Buffer preserved samples using flow cytometry analyses



#### Overview of experimental design

A.

Flow cytometry analyses were conducted on human PBMCs preserved in BD<sup>®</sup> OMICS-Guard Buffer and mouse splenocytes from tissue preserved in BD<sup>®</sup> **OMICS-Guard Buffer. Surface** protein expression and cell populations in both human PBMCs and mouse splenocytes were analyzed and compared to nonpreserved samples (controls) in a time-course study over 72 hours.

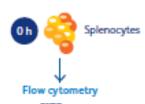




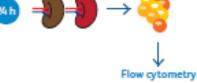


Not preserved Preserved 30-50 mg/20 mL

Spleen set 1 Spleen set 2

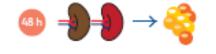






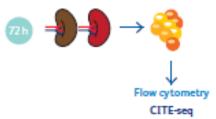
**BD<sup>®</sup> OMICS-Guard Buffer** 

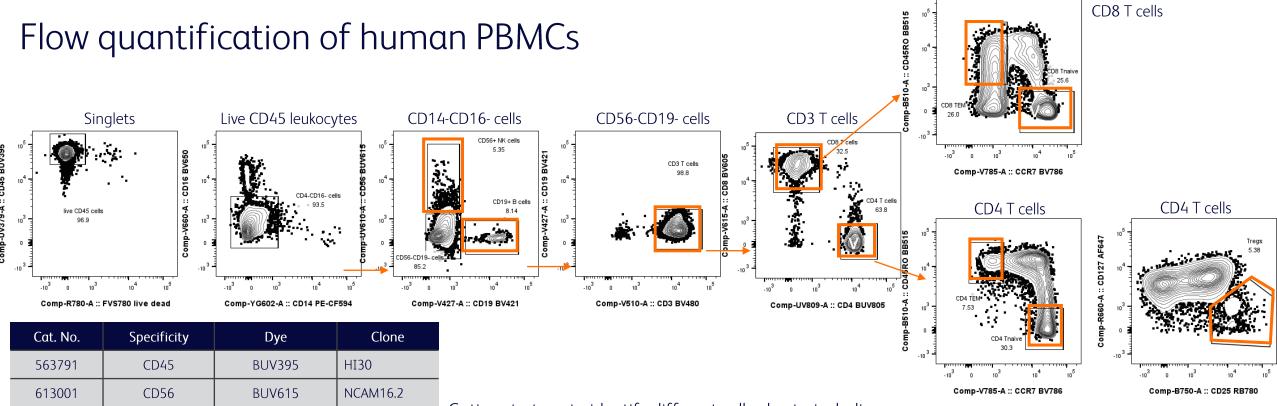
CITE-seq





CITE-seq





cat. Ho.	speemerey	Eje	cione
563791	CD45	BUV395	HI30
613001	CD56	BUV615	NCAM16.2
612887	CD4	BUV805	SK3
562440	CD19	BV421	HIB19
566105	CD3	BV480	UCHT1
564115	CD8	BV605	SK1
563691	CD16	BV650	3G8
566759	CCR7	BV786	2-L1-A
564530	CD45RO	BB515	UCHL1
562334	CD14	PE-CF594	МфР9
568689	CD25	RB780	2A3
560905	CD127	Alexa Fluor™ 647	HIL-7R-M21
565388	Live dea	Id FVS780 (APC-Cv7 ch	annel)

Gating strategy to identify different cell subsets, including:

- ➢ CD19 B cell
- CD56 NK cells
- CD4 TEM, CD4 naïve T cells, regulatory T cells, CD8 TEM, CD8 naïve T cells

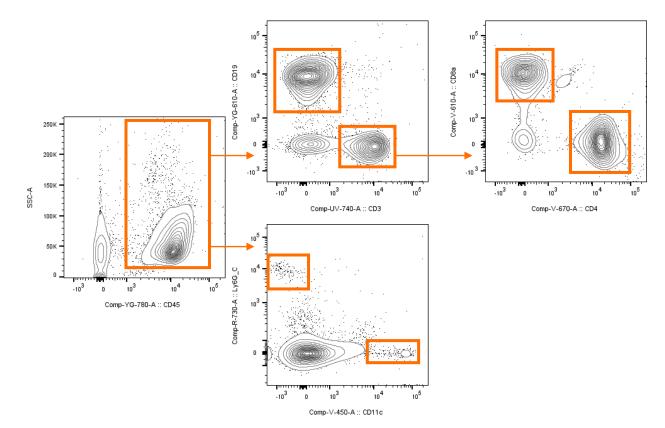
13-color fluorescent panel used for evaluating protein expression of human PBMCs across all time points. Contour figure plots show representative gating scheme to identify major live CD45+ cells types. Fluorescent antibodies were stained at optimal concentrations and labeling volumes in BD Pharmingen<sup>™</sup> Stain Buffer.

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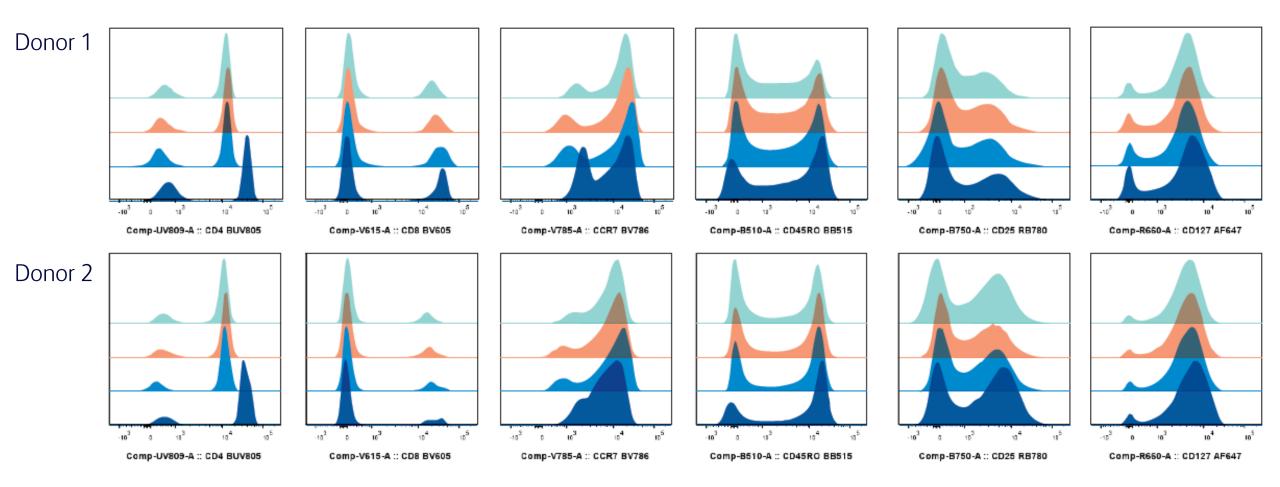
### Flow quantification of mouse splenocytes

Cat. No.	Specificity	Dye	Clone
741788	CD3	BUV737	17A2
740215	CD44	BUV395	IM7
562782	CD11c	BV421	HL3
563232	CD4	BV650	GK1.5
563152	CD8a	BV605	53-6.7
562291	CD19	PE-CF594	1D3
561868	CD45	PE-Cy7	30-F11
553271	CD43	PE	S7
567359	Ly6-G/C	R718	RB6-8C5
561919	CD62L	APC	MEL-14
561067	CD49b	FITC	DX5



11-color fluorescent panel used for evaluating protein expression of mouse splenocytes across all time points. Contour bivariate plots show representative gating scheme to identify major circulating leukocyte cells types and quantify surface protein expression. Fluorescent antibodies were stained at optimal concentrations and labeling volumes in BD Pharmingen<sup>™</sup> Stain Buffer.

#### Consistent protein expression over time in human PBMC samples

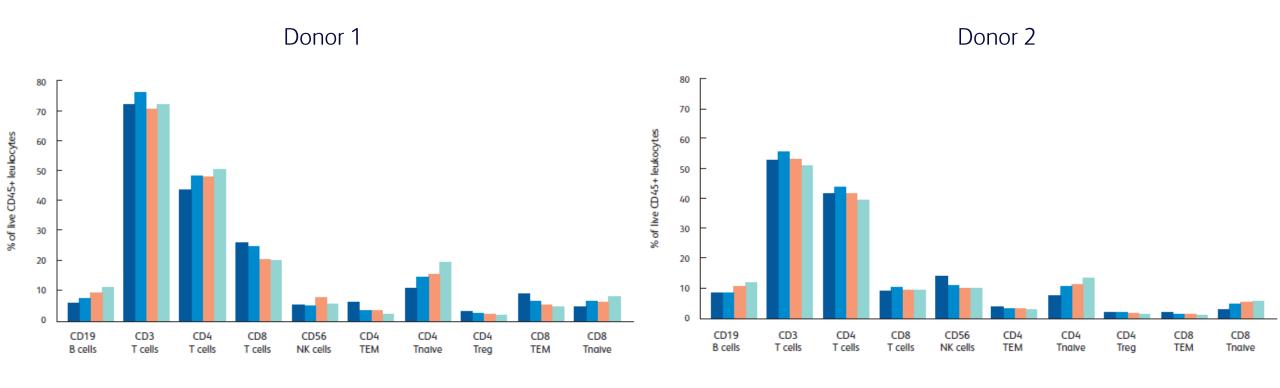


Flow histogram plots show consistent key protein marker expression represented by fluorescent signal between control (0 h) and preserved samples over time (24, 48, 72 h) for human PBMCs (top: Donor1, bottom: Donor 2). For both samples, CD14 and CD16 positive cells are excluded from live CD45+ leukocytes. NK cells and B cells are identified from CD14-CD16- cells. CD4 and CD8 T cells were gated from CD3 T cells. Effector memory T cells and regulatory T cells are evaluated on CD4 T cells.



72 h

#### Cell subpopulation frequencies of human PBMCs across preservation time points is consistent



📕 0 h 📕 24 h 📕 48 h 📕 72 h

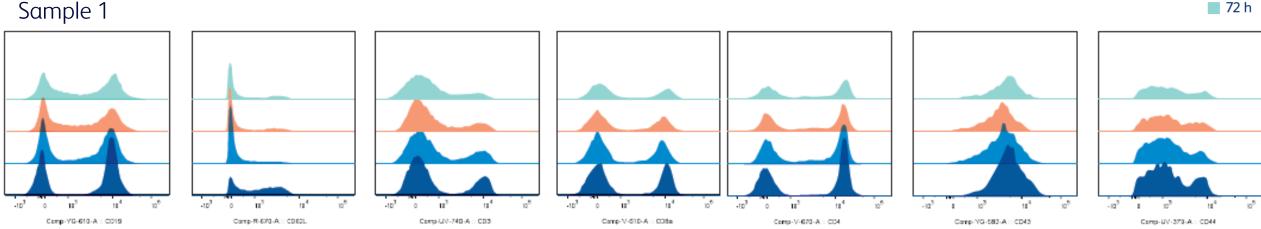
The ratio of different immune populations stays consistent over time for both Donor 1 and Donor 2. Proportions of live CD45+ cell types identified by corresponding cell surface marker(s), as outlined in the gating scheme, across donor and preservation time points.



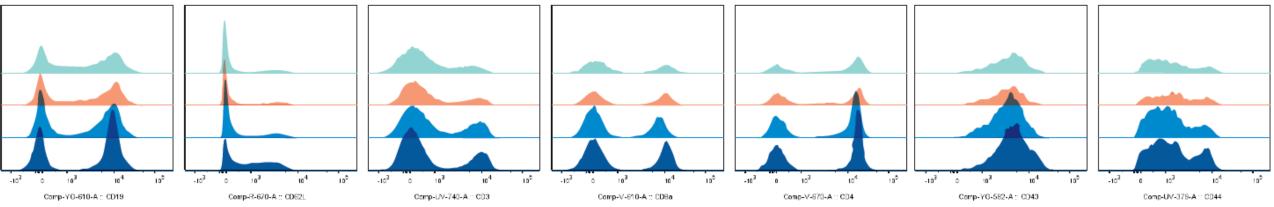
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#### Consistent protein expression over time in mouse spleen tissue samples



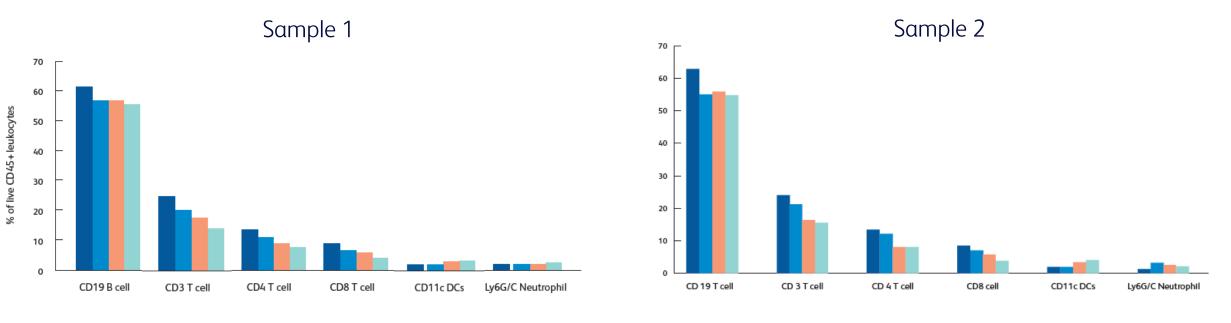


#### Sample 2



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 mouse spleen tissues (top: Sample 1, bottom: Sample 2). For both samples, CD19 and CD3 is gated on CD45+; CD62L is gated on CD19, and CD4 and CD8a histograms are from CD3 gate. Additionally, we show histograms for lymphocyte surface proteins CD43 and CD44 signal in CD4 event clusters. Cell type and surface markers signals are relatively consistent across donor and preservation time.

# Cell subpopulation frequencies of mouse splenocytes across preservation time points is consistent





The ratio of different immune populations stays consistent across time for both mouse spleen tissue samples. Proportions of major splenic leukocyte CD45+ cell types identified by corresponding cell surface marker, as outlined in the gating scheme, across sample and preservation time points.

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Cat. no.	Product description	
570908	BD® OMICS-Guard Sample Preservation Buffer Kit	
570911	BD® OMICS-Guard Sample Preservation Buffer	
Special Order	BD LSRFortessa™ X-20 Cell Analyzer	
Special Order	BD FACSymphony™ A5 SE Cell Analyzer	
555899	BD Pharm Lyse™ Lysing Buffer	
564220	BD Pharmingen™ Human BD Fc Block™ Reagent	E.c.
553141	BD Pharmingen™ Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™ Reagent)	
554656	BD Pharmingen™ Stain Buffer (FBS)	

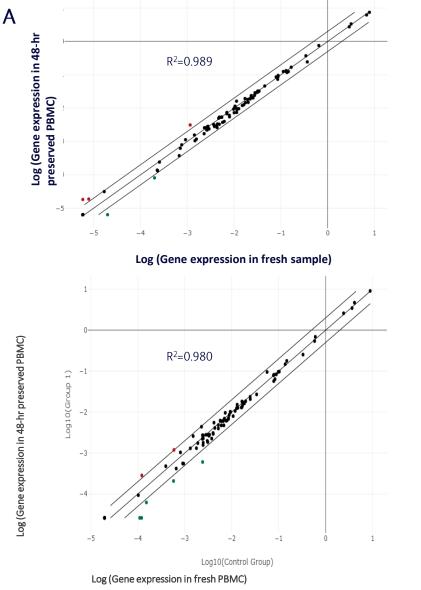


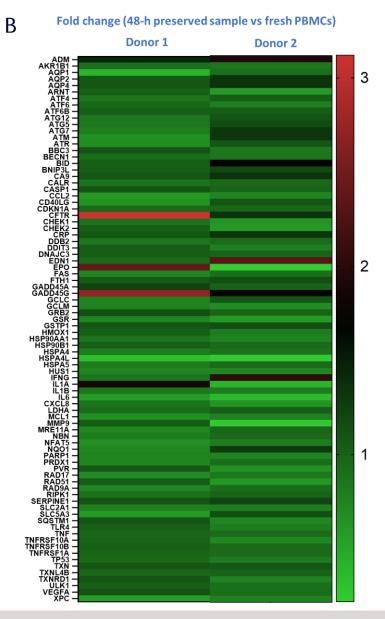
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### Gene expression analysis using qPCR with BD® OMICS-Guard Buffer preserved samples



#### BD<sup>®</sup> OMICS-Guard Buffer preservation workflow induces minimum cell stress





In a separate set of experiments, to evaluate the impact of BD<sup>®</sup> OMICS-Guard Buffer on cellular stress pathways, a qPCR study was performed wherein expression of stressassociated genes was compared between fresh and BD<sup>®</sup> OMICS-Guard Buffer-preserved PBMC samples. Results revealed no significant expression change of stress-associated genes after 48 h of preservation.

RNA was isolated from fresh and 48-h preserved human PBMCs from two donors using the RNeasy<sup>™</sup> Mini Kit (Qiagen). RIN (RNA integrity number) was evaluated using an Agilent TapeStation System after RNA isolation. cDNA was produced using the RT2<sup>™</sup> First Strand Kit (Qiagen), and bulk gene expression of 84 stress-associated genes was analyzed using the RT<sup>2</sup> Profiler<sup>™</sup> 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 R2>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.

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**BD** 

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Cat. no.	Product description	
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570911	BD® OMICS-Guard Sample Preservation Buffer	

# Thank you



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