BD® OMICS-Guard Sample Preservation Buffer preserves mRNA and cell surface epitopes for single-cell sequencing applications

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Abstract

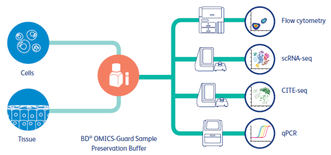
Due to the sensitivity of sequencing techniques, sample quality and stability can potentially interfere with results. BD® OMICSGuard Sample Preservation Buffer (SPB), a novel preservation solution, allows cells and bulk tissues to be stored for up to 72hr at 4 °C, while reliably capturing the true biology of cells. This reagent gently preserves mRNA and protein integrity without traditional cross-linking and harsh fixatives, increasing flexibility and expanding options for study designs. Here, we showcase the preservation properties of this reagent for multiomic readouts in human cells and mouse tissue across three timepoints. SPB preservation of mRNA from human PBMC and bulk mouse spleen tissues is shown by correlation graphs of differentially expressed genes in preserved samples compared to fresh/unpreserved controls. Protein epitope preservation was demonstrated via CITE-Seq assay with the BD® AbSeq Human Immune Discovery Panel (PBMC) or a 30-plex panel of anti-mouse BD® AbSeq Antibody-Conjugated Oligonucleotides (mouse splenocytes) and confirmed with multicolor flow cytometry. For both sample types, high correlation R2 values (>0.87) were calculated for the preservation time points versus fresh controls. Flow cytometry analysis of major cell types and surface protein expression was consistent with sequencing data for all samples. Specificity and sensitivity of major cell type markers and cell surface proteins across cell types and time is visualized by BD® AbSeq Antibody-generated heatmap for each sample. BD® SPB maintains mRNA integrity in targeted gene assays and VDJ full-length assays, suggesting robust preservation of select transcripts readily interrogated in single-cell profiling. Batch effect across time and technical replicates for both sample types was limited with the BD Rhapsody™ HT Xpress System and delineated by donor/sample specific tSNEs across time.

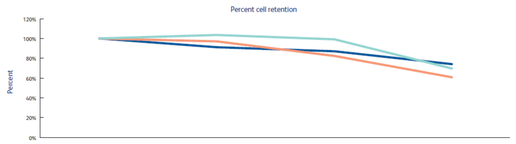
We demonstrate that the BD® OMICS-Guard Sample Preservation Buffer effectively preserves both mRNA and surface proteins for single-cell experiments. BD® OMICS-Guard SPB allows the convenient preservation of samples such as tumor biopsies to be collected and stored for up to 72hr at 4 °C while maintaining mRNA and protein integrity, enhancing flexibility for your precious samples.

Key Features of BD® OMICS-Guard Buffer

"Protect your samples, guard your science"

- PFA-free reagent that mildly "fixes" cells while maintaining mRNA and cell surface epitope integrity
- Protects and preserves samples for up to 72 hours at 4 °C
- Hassle-free with a simple one-step preservation protocol yielding high cell retention (see below)
- No bias in cell type preservation, conserves cell-type distribution
- Can be used with both cell suspensions and tissue samples



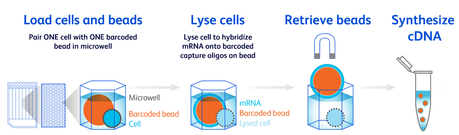


	0 h	24 h	48 h	120 h
1 million	100%	91%	87%	74%
0.1 million	100%	97%	82%	61%
10 million	100%	104%	99%	70%

Cell retention rate for fresh (not previously frozen) human PBMCs was determined for three different cell amounts: 0.1 M, 1 M and 10 M stored in 1 mL of BD® OMICS-Guard Buffer for up to 120 hrs. The number of cells were normalized to the control sample at 0 hr.

Application of BD® OMICS-Guard Sample Preservation Buffer with the BD Rhapsody System

The BD Rhapsody™ HT Xpress System is a higher-throughput platform that leverages our proprietary, gentle, robust microwell-based single-cell partitioning technology to perform single cell analysis. The 8-lane system facilitated the testing of preservation over time with just one cartridge.



Experimental Scheme

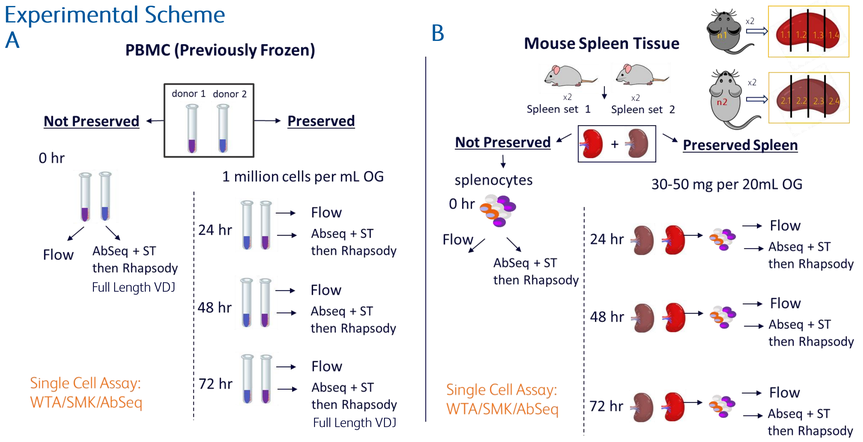
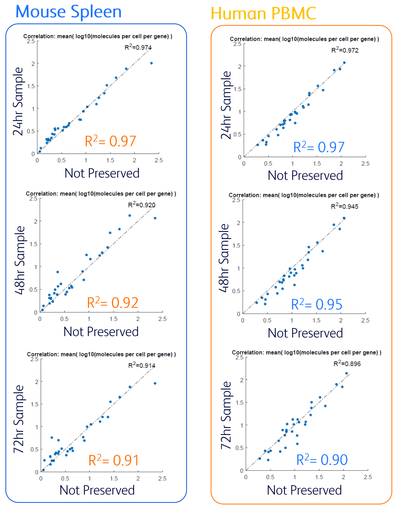


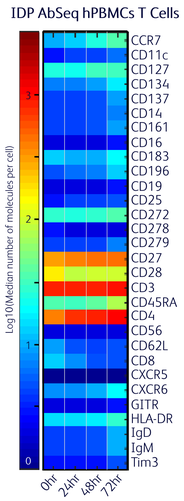
Figure 1: Time course experiment using previously frozen human PBMCs and fresh mouse spleen tissue preserved with OMICS-Guard for 24, 48 and 72 hours at 4C and compared to non-preserved samples at Day 0 (0 hour). A portion of cells and tissue were processed by flow cytometry as well as through the BD Rhapsody Single Cell analysis system. For the Rhapsody single cell workflow, samples were stained with AbSeq and multiplexed using the BD Sample Multiplexing kit (SMK) to combine various donors or mice tissue into a single lane on a cartridge. Following the cartridge workflow, beads were subsampled and processed using a Whole Transcriptome (WTA) + AbSeq + SMK assay. Additionally, WTA + Full Length VDJ + AbSeq was processed for 0hr fresh control and 72hr sample. (A) Previously frozen human PBMCs from 2 different donors were stored in OG at a concentration of approximately 1 million cells per mL of OG over the course of 72 hours at 4C. Each donor was stored in parallel, and a portion of the cells were removed at various time points. Cell were stained with either a 13-plex fluorophore antibody panel or co-stained with a 30-plex BD Immune Discovery AbSeq Panel (IDP) and SMK. A portion of cells was not preserved and used as a control on Day 0 (0hr). (B) Spleens from two sets of 2 mice were harvested and equally portioned into 4 sections as shown in the image. Approximately 30-50 mg of aseptically harvested tissue from 2 mice were portioned and combined and preserved in 20 mL of OG, which constituted 1 set of spleens. A different set of mice were also processed and preserved the same way in parallel and constituted as set 2. After preservation, splenocytes were extracted and stained with either a 11-plex fluorophore antibody panel or co-stained with a 30-plex mouse AbSeq Panel and mouse SMK. A portion of tissue was not preserved and used as a control on Day 0 (0hr).

Consistent protein expression between preserved and fresh samples

3A Correlations of 30-plex AbSeq



3B BD IDP heatmap



3C Flow analysis of OG preserved human CD4+ cells

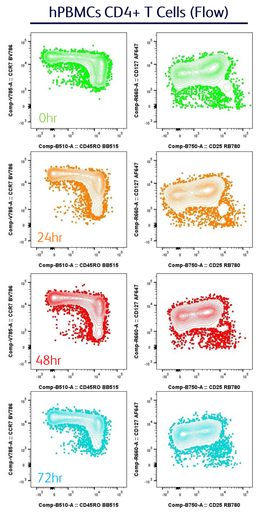
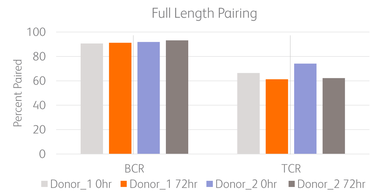


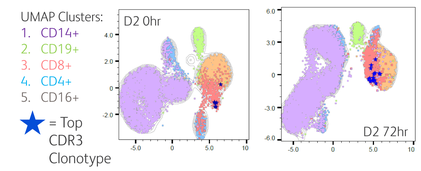
Figure 3: AbSeq correlation plots to evaluate the preservation of cell surface epitopes. For mouse spleen a custom 30-plex AbSeq panel was used. Human PBMCs were stained with BD IDP 30-plex panel. BD Abseq Enhancer was utilized for all Abseq experiments. AbSeq expression was compared between the non-preserved control (0 hr) sample and the 24, 48 and 72 hr time points (A), for mouse spleen and representative human PBMC donor 1. R^2 correlation values were calculated for each plot, plots were generated with BD DataView. Sequencing data were normalized to the same read-depth followed by demultiplexing of samples. (B) Representative Abseq heatmap for human donor 1 CD3 T cells at time points (0, 24, 48, and 72 hr) shows to specificity AbSeq in preserved cells, no expression of CD19 and IgM in T cells as expected, and BD AbSeq Enhancer reduced background noise. (C) 13 color fluorescent panel was used to evaluating protein expression of representative human fresh and preserved PBMCs, figure depicts contour plot expression of cell surface proteins on CD4+ T cells.

Preservation for Full Length VDJ Assay

4A Similar pairing efficiency in preserved vs fresh samples in BD Full Length VDJ Assay



4B Top TCR clonotype is conserved between fresh and preserved sample



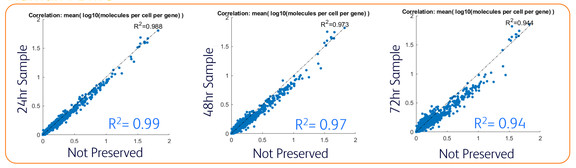
TCR_Alpha SQQGEEDPQALSIQEGENATMNCSYKTSINNLQWYRQNSGRGLVHLILI RSNEREKHSGRLRVTLDTSKKSSSLLITASRAADTASYFCATDGTDNTDKLI ATDGTDNTDKLI

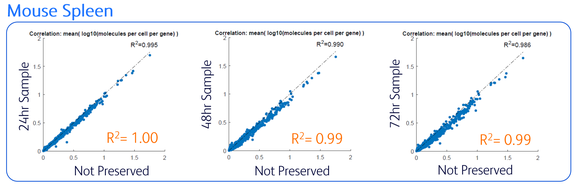
TCR_Beta EAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYY SMNVEVTDKGDVPEGYKVSRKEKRNFPLILESPSPNQTSLYFCASSLRRNE KLFFGSGTQLSVL

4A Consistent pairing rate for BCR/TCR between 0hr control and 72hr preserved samples is indicative of preservation stability. **4B** Clonotype frequency analysis with BD SeqGeq identified the conservation of the top clonotype, within the same donor, for fresh control and preserved sample. Top clonotype is overlayed with cell cluster UMAP. The full-length amino acid sequence of the V(D)J alpha and beta TCR chains of the top clonotype is annotated showing framework and CDR regions, in corresponding colors.

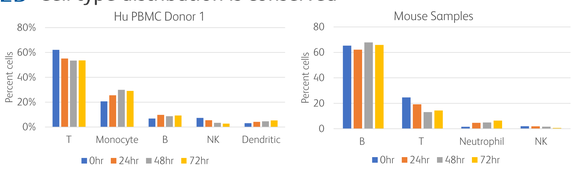
mRNA is well preserved with BD® OMICS-Guard

2A scWTA mRNA correlation for human and mouse samples





2B Cell type distribution is conserved



2C Mouse neutrophil transcriptome is preserved for up to 72hr

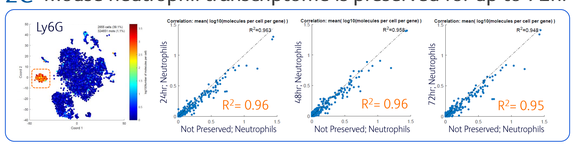


Figure 2: (A) Whole transcriptome correlation plots. R^2 correlation values were calculated using differentially expressed gene plots from WTA data, for representative donor of human PBMC and mouse spleen. Each plot compares the non-preserved control (0 hr) sample with preserved time points. Sequencing data were normalized to the same read-depth. (B) Cell type distribution is maintained in cells across preservation time points, compared to 0hr control. Cell types profile in human PBMCs (A) was determined through mRNA via BD Bioinformatics pipeline. Mouse spleen cell type calling was used gene expression markers to guide manual annotation. E. Spleen resident neutrophil clusters, determined by Ly6G expression, were annotated and R^2 correlation value between the preserved time points and fresh control for were calculated.

Conclusions

- BD® OMICS-Guard (OG) Sample Preservation Buffer maintains mRNA integrity and cell surface epitopes for up to 72 hours at 4°C.
- OG has an easy-to-use protocol with minimal hands-on time and can be used with single cell applications or flow cytometry
- The percent of cells recovered after preservation is high even after 72 hours in OG.
- Consistent TCR/BCR pairing rate between fresh and preserved samples in Full-Length VDJ assay
- There are no major shifts in the distribution of cell types after preservation in OG, even with fragile cells such as neutrophils

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