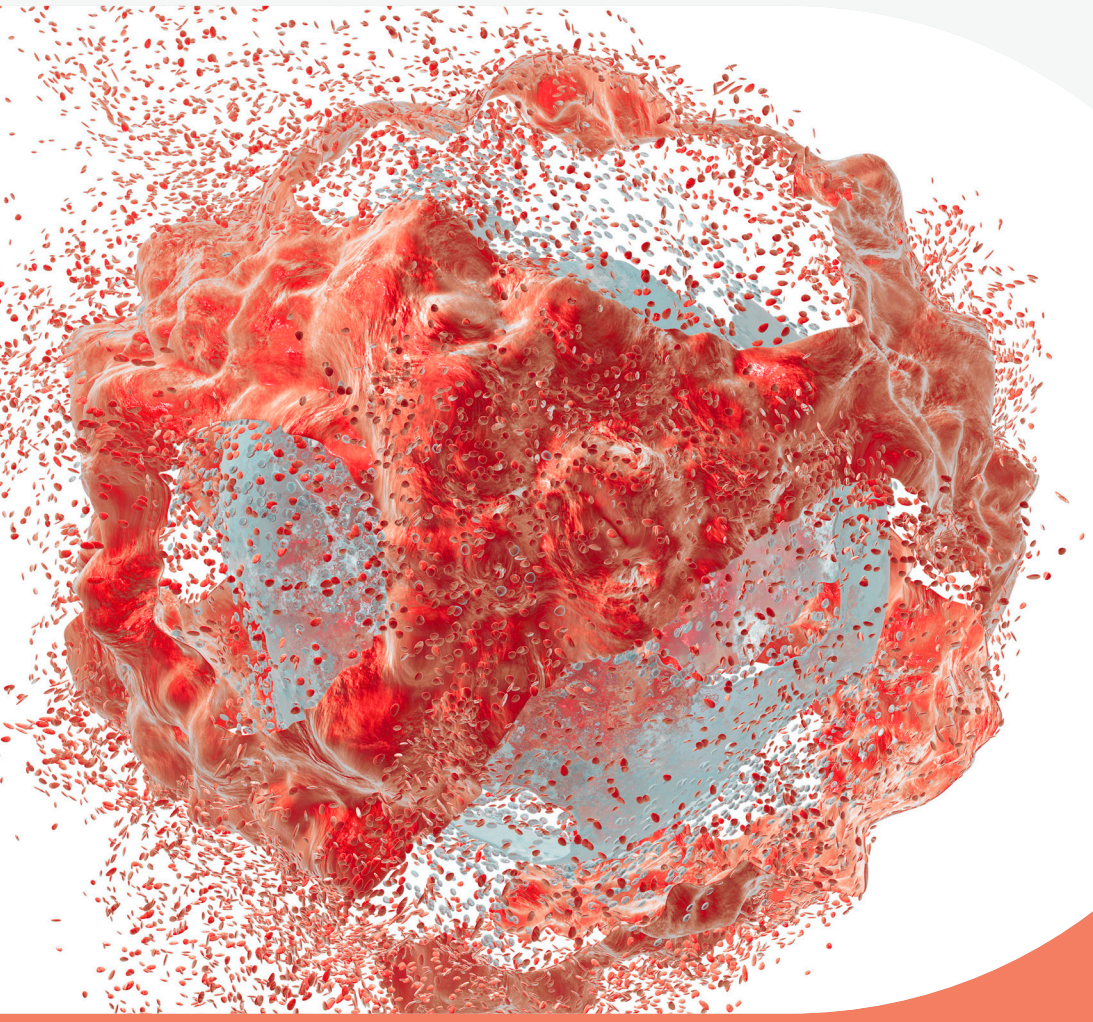


Intracellular CITE-seq Assay using BD® AbSeq Antibody-Oligos

Redefine CITE-seq: Expand the power of CITE-seq to intracellular proteomics

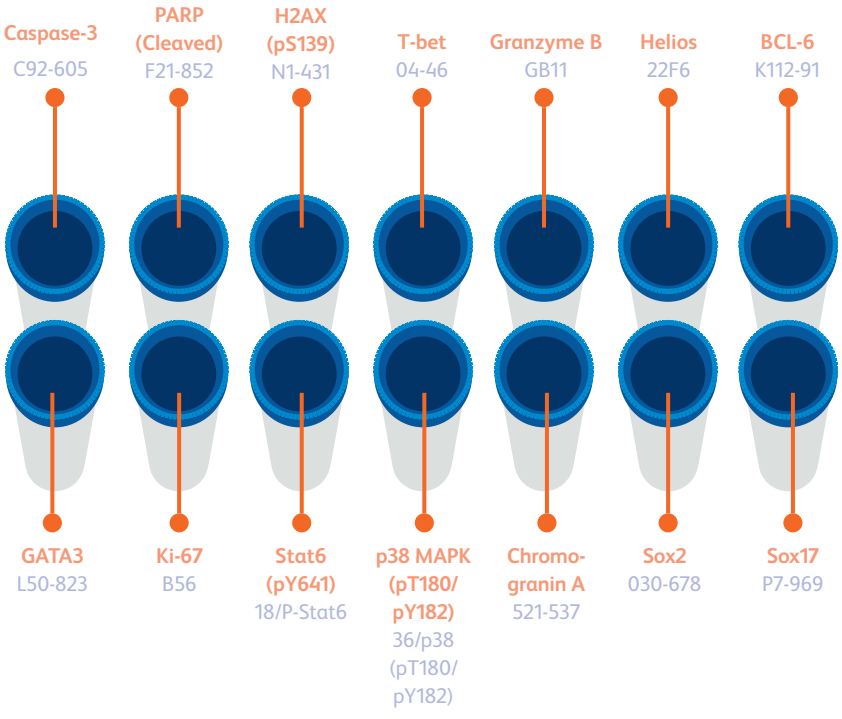
CITE-seq facilitates understanding of cell heterogeneity and single-cell phenotyping primarily focusing on cell surface protein expression.[1] CITE-seq with insights into intracellular protein expression (intracellular CITE-seq or IC CITE-seq) enables a more comprehensive view of biological drivers of cellular functions and signaling responses. The Intracellular CITE-seq Assay using BD® AbSeq Antibody-Oligos offers a complete solution for whole transcriptome and surface and intracellular protein detection with a validated protocol and intuitive bioinformatics pipeline for data analysis.

The Intracellular CITE-seq Assay using BD® AbSeq Antibody-Oligos

- Enables simultaneous RNA, surface protein and intracellular protein detection
- Works with the BD® Single-Cell Multiplexing Kit to enable sample multiplexing
- Supports high-plex proteomics profiling (~100 protein markers) at the single-cell level
- Drives deeper understanding of cellular functions such as signal transduction dynamics and transcriptional regulation
- Allows a safe stopping point during the workflow without compromising assay sensitivity

Available intracellular BD® AbSeq Antibody-Oligos

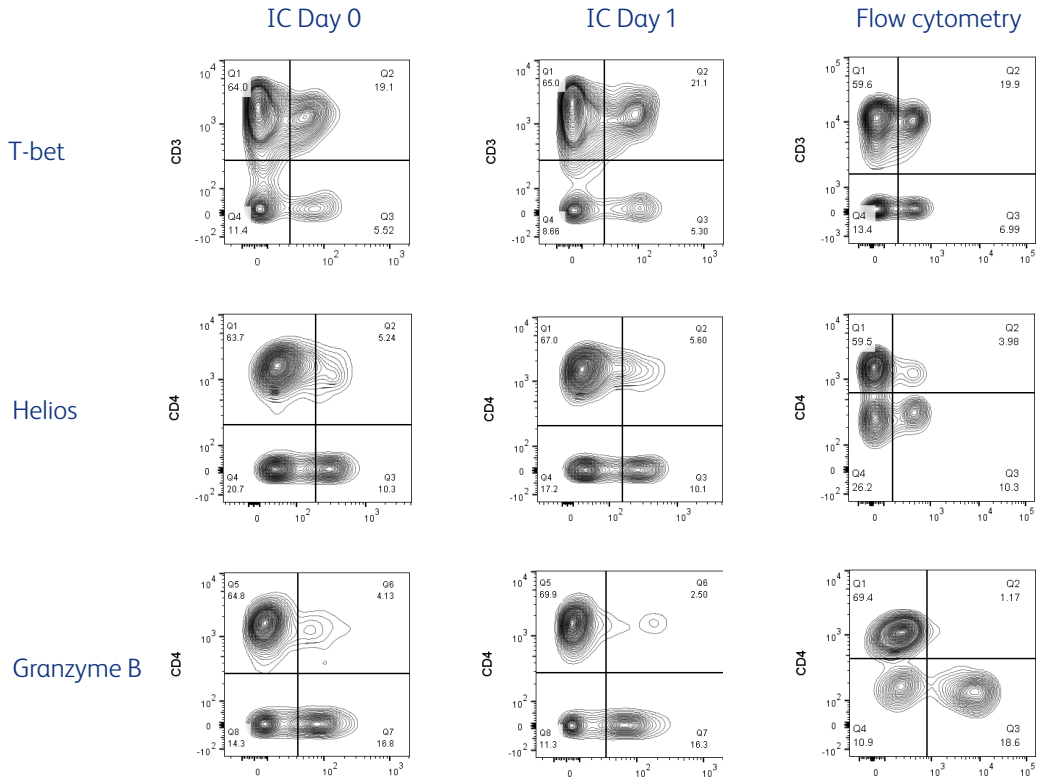
Each intracellular BD® AbSeq Ab-Oligo has been validated in an optimized model system, is pre-titrated to optimal concentration, and is offered in a 25 tests per vial format.



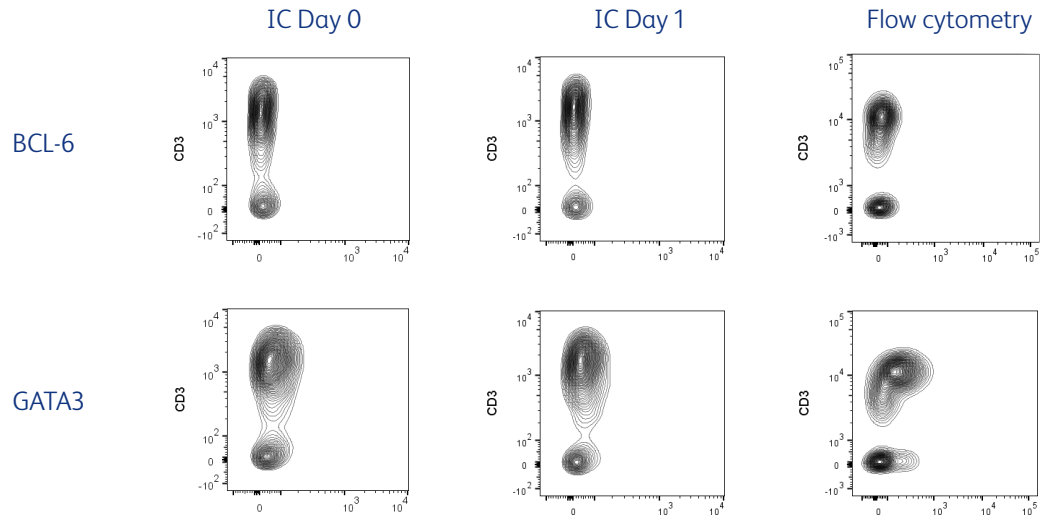
Detect intracellular protein expression with IC CITE-seq using BD® AbSeq Ab-Oligos

The Intracellular CITE-seq Assay using BD® AbSeq Ab-Oligos facilitates accurate quantification of target proteins. When compared to well-established intracellular flow cytometry, high concordance of intracellular protein expression was observed between intracellular BD® AbSeq Antibody-Oligos and flow cytometry.

A. Intracellular protein markers expected to be expressed in resting PBMCs



B. Intracellular protein markers not expected to be expressed in PBMCs



C.

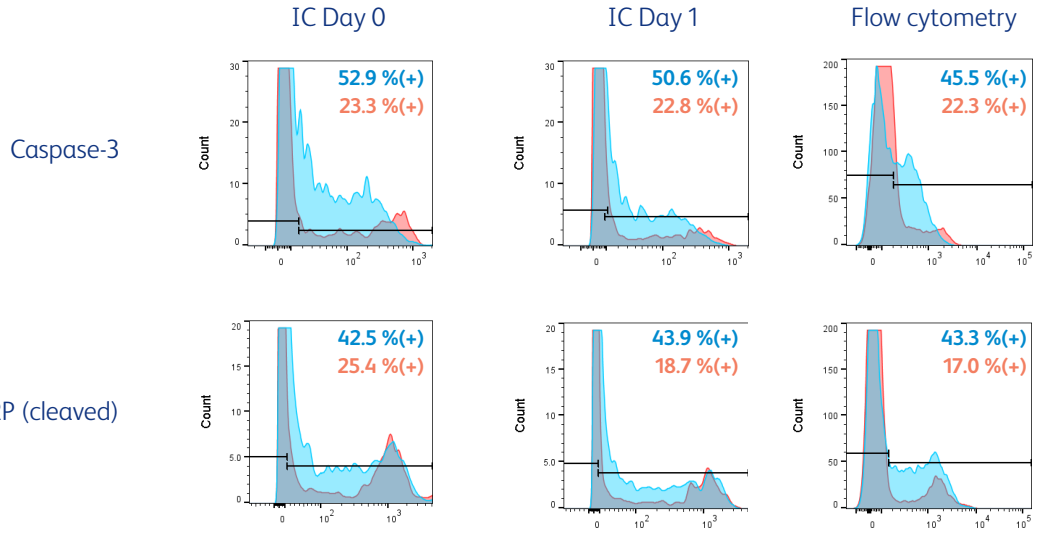


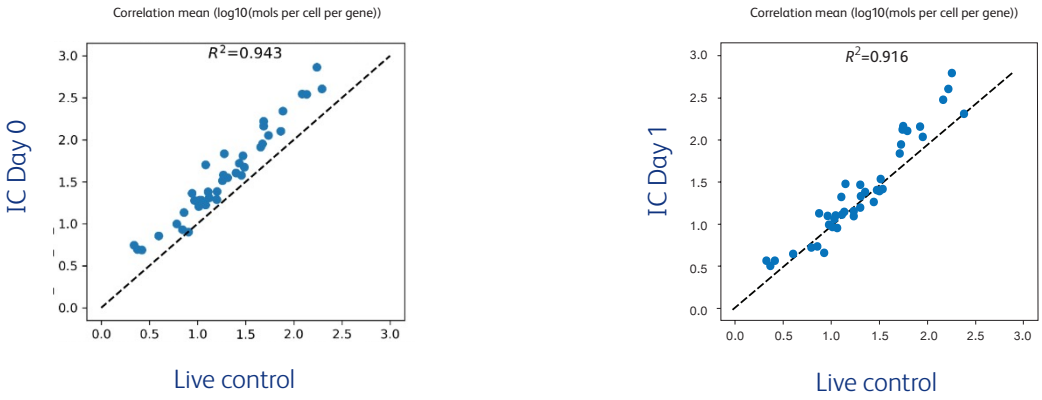
Figure 1. Detection of intracellular protein expression by IC CITE-seq using BD® AbSeq Ab-Oligos and flow cytometry.

Resting and PMA- and ionomycin-stimulated human PBMCs were stained with a 49-plex BD® AbSeq Panel including 40 surface markers and nine intracellular markers following the 5-min preservation workflow (IC Day 0, n = 3) and 24-h preservation workflow (IC Day 1, n = 1). The IC-treated PBMC samples were compared to live controls stained with the same 40-plex surface BD® AbSeq Panel (n = 2) as well as flow cytometry controls stained with the same intracellular antibodies. Figures show expression patterns of representative intracellular protein markers profiled by intracellular BD® AbSeq Antibody-Oligos and flow cytometry (left column: IC Day 0, middle column: IC Day 1, right column: Flow cytometry). Intracellular BD® AbSeq Antibody-Oligos show consistent performance when cells are preserved for 5 min or 24 h and are in high concordance with flow cytometry results. **A)** T-bet, Helios and Granzyme B are expected to be expressed in subsets of resting PBMCs. **B)** Bcl-6 and GATA3 are not expected to be expressed in PBMCs. **C)** Apoptosis markers Caspase-3 and PARP (cleaved) are expected to be expressed in stimulated PBMCs.

Profile surface proteins with high confidence while detecting intracellular proteins

Preserving cell surface epitopes during the permeabilization process in an intracellular protein analysis workflow is critical. The Intracellular CITE-seq Assay using BD® AbSeq Antibody-Oligos has been optimized to ensure the integrity of cell surface protein epitopes during the workflow and our data demonstrate that surface protein expression is not affected by the intracellular staining process.

A.



B.

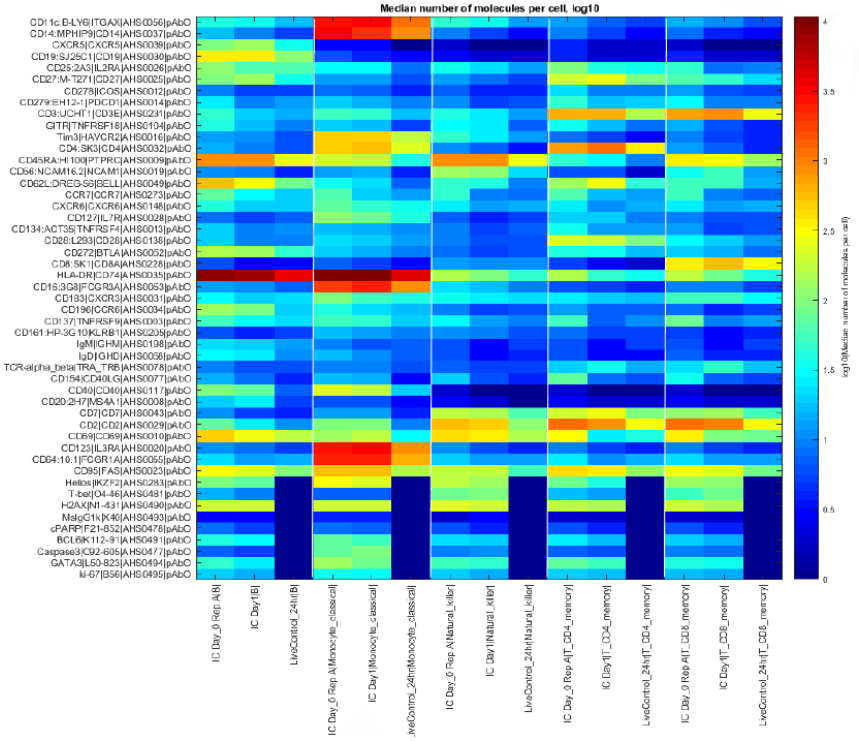


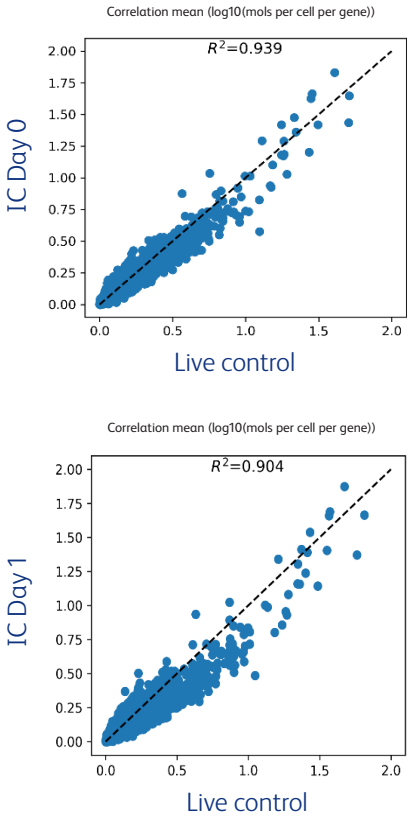
Figure 2. Surface BD® AbSeq Ab-Oligo expression and sensitivity are highly correlated between IC-treated samples and live controls.

Study carried out as previously described in Figure 1. **A)** Correlation of surface BD® AbSeq Ab-Oligo expression between IC Day 0 (left) vs live control and IC Day 1 (right) vs live control (both R^2>0.9). **B)** Sensitivity of surface BD® AbSeq Ab-Oligos represented by median molecules per cell of each BD® AbSeq Ab-Oligo in major PBMCs (B cells, classical monocytes, NK cells, CD4 and CD8 T-cells). Cell type annotation was performed based on gene expression.

Transcriptome analyses with the Intracellular CITE-seq Assay using BD® AbSeq Antibody-Oligos

The Intracellular CITE-seq Assay using BD® AbSeq Antibody-Oligos features high transcriptome detection sensitivity. High gene expression correlation and recovery of up to 90% WTA sensitivity was observed between non-IC treated and IC-treated samples. These data demonstrate the ability of the Intracellular CITE-seq Assay to preserve transcriptomic information.

A.



B. WTA analysis pipeline metrics

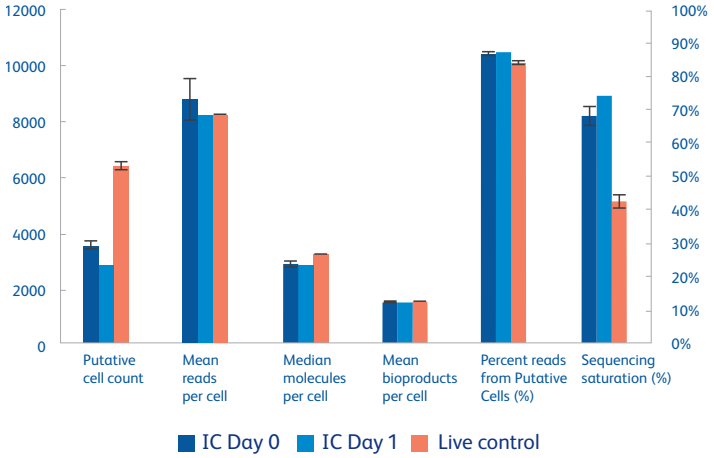


Figure 3. Gene expression and WTA assay sensitivity are highly correlated between IC-treated samples and live controls.

Study carried out as previously described in Figure 1. **A)** Gene expression correlation between IC Day 0 (top) vs live control and IC Day 1 (bottom) vs live control (both R^2>0.9). **B)** Cell-capture beads were subsampled targeting 5,000 cells for sequencing. WTA assay sensitivity represented by median molecules per cell and median genes per cell were compared between IC-treated samples (IC Day 0 and IC Day 1) and live controls. IC Day 0 and IC Day 1 samples recovered ~90% of WTA assay sensitivity of live controls at similar sequencing reads per cell.

The Intracellular CITE-seq Assay using BD® AbSeq Antibody-Oligos preserves major cell populations

Specific cell groups are susceptible to the harsh cell fixation and permeabilization conditions in traditional intracellular proteome analyses. The Intracellular CITE-seq Assay using BD® AbSeq Antibody-Oligos protects cell composition and ensures recovery of major cell populations in single-cell samples.

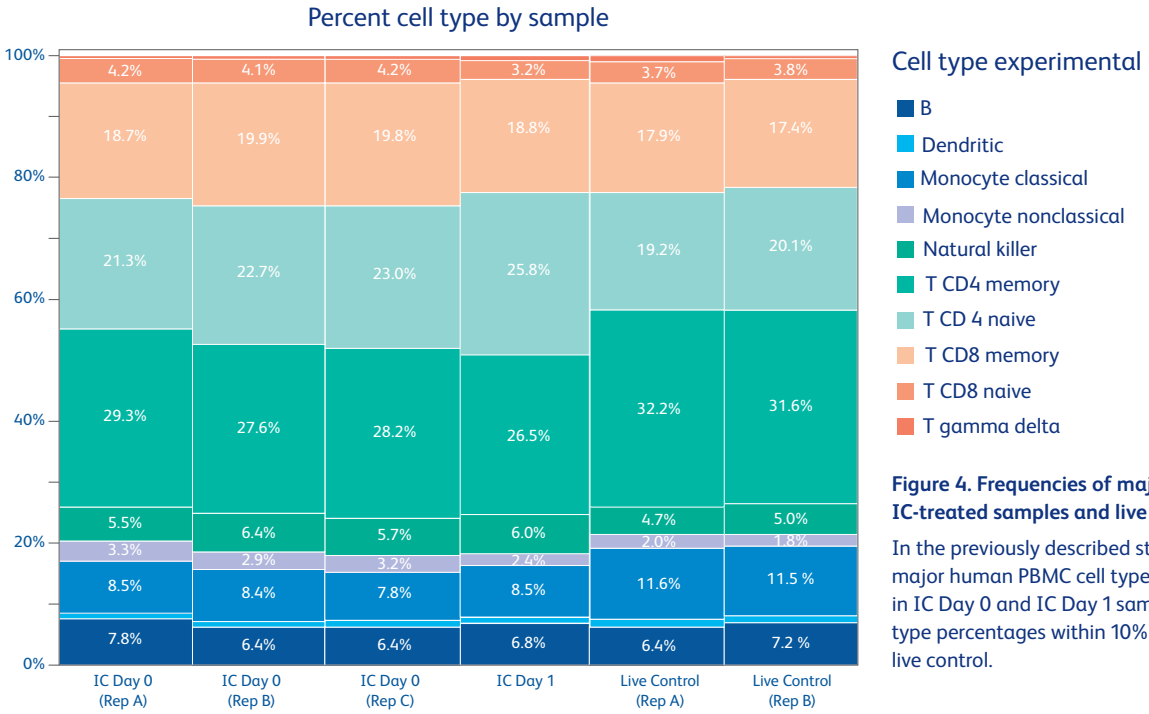


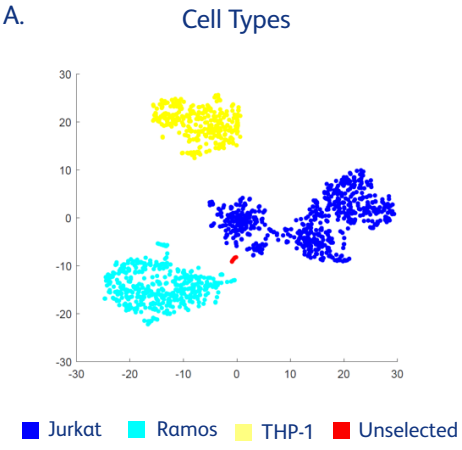
Figure 4. Frequencies of major cell populations in IC-treated samples and live controls.

In the previously described study in **Figure 1**, major human PBMC cell types were identified in IC Day 0 and IC Day 1 samples, showing cell type percentages within 10% variation of total of live control.

Sample multiplexing is enabled with the Intracellular CITE-seq Assay using BD® AbSeq Antibody-Oligos

The Intracellular CITE-seq Assay using BD® AbSeq Antibody-Oligos can work seamlessly with the BD® Single-Cell Multiplexing Kit to increase sample throughput, reduce experimental cost and, most importantly, reduce batch effects while maintaining high sample multiplexing specifications.

A.



B.

	Specificity	Sensitivity
Jurkat	92%	99%
Ramos	100%	99%
THP-1	98%	99%

Figure 5. High sample multiplexing specificity and sensitivity with the Intracellular CITE-seq Assay.

Stimulated Jurkat cells treated with camptothecin and resting Jurkat, Ramos and THP-1 cell lines were co-stained with the BD® AbSeq Immune Discovery Panel (IDP) and BD® Human Single-Cell Multiplexing Kit (SMK) Sample Tags 1, 2, 3 and 4, respectively. The surface-stained samples were pooled and stained with intracellular BD® AbSeq Antibody-Oligos (PARP (cleaved), BCL-6, Caspase-3, GATA3, H2AX) and analyzed with a WTA assay. For the purposes of calculating sample multiplexing specificity and sensitivity, results from the resting and stimulated Jurkat cells were calculated as one cell line because they were difficult to differentiate in silico. Human SMK specificity and sensitivity detected for all cell lines was greater than 90%, indicating that the IC treatment did not affect sample multiplexing performance. **A)** tSNE plot showing cell cluster based on mRNA gene expression and cell type annotation based on Samples Tags (Jurkat is combination of Sample Tag 1 and Sample Tag 2). **B)** High specificity and sensitivity of human SMK for each cell type.

Product purchase guide for the Intracellular CITE-seq Assay using BD® AbSeq Antibody-Oligos

The Intracellular CITE-seq Assay requires reagents in addition to those needed for CITE-seq with the BD Rhapsody™ Whole Transcriptome Analysis Assay and surface BD® AbSeq Antibody-Oligos.

WTA assay

- BD Rhapsody™ Enhanced Cartridge Reagent Kit
- BD Rhapsody™ Cartridge Kit or 8-Lane Cartridge
- BD Rhapsody™ cDNA Kit
- BD Rhapsody™ WTA Amplification Kit

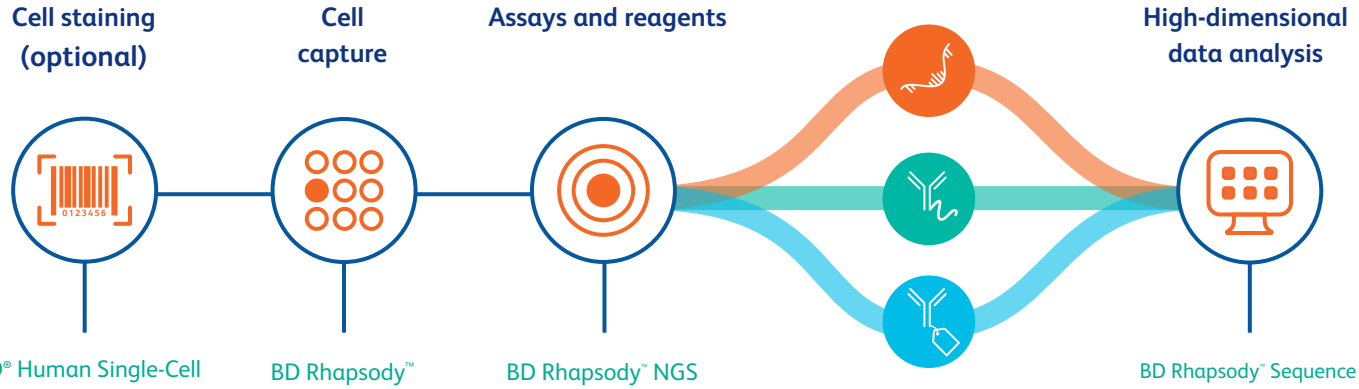
+

Additional reagents required

- BD Rhapsody™ Intracellular AbSeq Buffer Kit
- BD® RNase Inhibitor
- BD® AbSeq Enhancer Kit
- BD® OMICS-Guard Sample Preservation Buffer

+

Order the single-vial surface and intracellular BD® AbSeq Antibody-Oligos separately to suit your experimental needs



Cell staining (optional)

BD® Human Single-Cell Multiplexing Kit

x12 sample tags

BD® AbSeq Antibody-Oligonucleotides

Surface BD® AbSeq Ab-Oligos

Intracellular BD® AbSeq Ab-Oligos

BD® AbSeq Immune Discovery Panel

Custom BD® AbSeq Ab-Oligos

Cell capture

BD Rhapsody™ HT Xpress System

Single-cell capture, bead-based mRNA isolation and bead retrieval

BD Rhapsody™ HT Single-Cell Analysis System

Single-cell capture, bead-based mRNA isolation, bead retrieval with imaging and cell workflow QC

Assays and reagents

BD Rhapsody™ NGS Library Prep Kits

BD Rhapsody™ Whole Transcriptome Analysis Amplification Kit

High-dimensional data analysis

BD Rhapsody™ Sequence Analysis Pipeline

Free intuitive and scalable software for bioinformatics analyses

Ordering information

Description	Cat. No.
BD Rhapsody™ Intracellular AbSeq Buffer Kit	570742
BD® AbSeq Enhancer Kit	570750
BD® OMICS-Guard Sample Preservation Buffer Kit	570908
BD® RNase Inhibitor	570751
Helios Oligo AHS0283 22F6 25Tst	940509
Sox2 Oligo AHS0332 O30-678 25Tst	940510
Sox17 Oligo AHS0471 P7-969 25Tst	940511
Chromogranin A Oligo AHS0475 S21-537 25Tst	940512
Caspase-3 Oligo AHS0477 C92-605 25Tst	940513
PARP Oligo AHS0478 F21-852 25Tst	940514
T-bet Oligo AHS0481 O4-46 25Tst	940515
Stat6 Oligo AHS0482 18/P-STAT6 25Tst	940516
Granzyme B Oligo AHS0487 GB11 25Tst	940517
p38 MAPK Oligo AHS0489 36/P38 25Tst	940518
H2AX Oligo AHS0490 N1-431 25Tst	940519
BCL-6 Oligo AHS0491 K112-91 25Tst	940520
GATA3 Oligo AHS0494 L50-823 25Tst	940521
Ki-67 Oligo AHS0495 B56 25Tst	940522
Suggested companion products	
BD Rhapsody™ HT Xpress System Package	666625
BD Rhapsody™ Scanner	633701
BD Rhapsody™ Whole Transcriptome Analysis (WTA) Amplification Kit	633801
BD Rhapsody™ cDNA Kit	633773
BD Rhapsody™ Enhanced Cartridge Reagent Kit	664887
BD Rhapsody™ 8-Lane Cartridge	666262
BD® AbSeq Immune Discovery Panel	625970
BD® Human Single-Cell Multiplexing Kit	633781
BD® AbSeq Single-Vial Reagents	Contact for more info*
BD Pharmingen™ Human BD Fc Block™ Reagent	564219
BD Pharmingen™ Stain Buffer (FBS)	554656

*To request a quote or place an order, visit **bdbiosciences.com/scM-reagents**, email **scomix@bd.com** or contact your local BD sales representative.

References

1 Stoeckius M, Hafemeister C. Stephenson W, et al. Simultaneous epitope and transcriptome measurement in single-cells. *Nat Methods*. 2017;14(9):865-868. doi: 10.1038/nmeth.4380

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