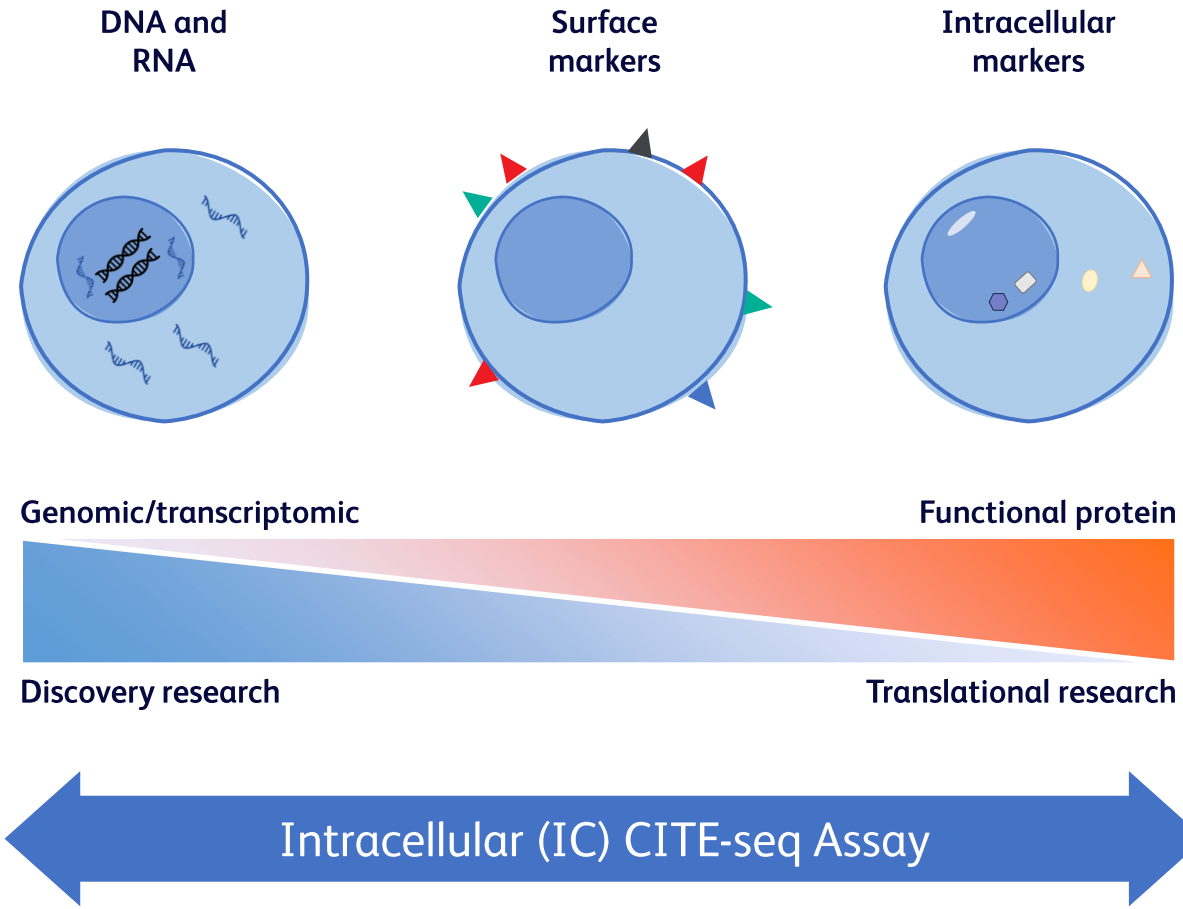
Intracellular CITE-seq using BD® AbSeq Antibody-Oligos

IC CITE-seq Assay presentation

BD

Gain deeper multiomic insights—Intracellular protein + cell surface protein + RNA



- Simultaneous single-cell transcriptomic, surface and intracellular proteomic analysis with NGS readout
- Enables a holistic understanding of cellular dynamics such as transcription regulation and functional states
- Decreases data acquisition time and conserves precious samples

Redefine CITE-seq: Profile intracellular proteins, surface proteins and RNA in one single-cell experiment

Single-cell multiomics

- Simultaneously detect single-cell **transcriptome, surface and intracellular proteome** profiles
- Yields high WTA sensitivity* for RNA analyses
- Faithfully recover expected cell subsets in your sample

High-parameter proteomics profiling

- Profile up to 100 protein markers including surface and intracellular proteins in a single experiment

Sample multiplexing enabled

- Multiplex up to 12 samples using the BD® Single-Cell Multiplexing Kit (SMK)

Validated assay

- Fully validated on the BD Rhapsody™ System
- Each IC BD® AbSeq Ab-Oligo is validated against flow cytometry controls
- Comparable IC protein performance vs flow cytometry

Simple and free bioinformatics analysis

- Analyze data with the intuitive BD Rhapsody™ Sequence Analysis Pipeline
- Same pipeline for analysis of intracellular and surface proteins

Efficient and flexible workflow

- ~2 hours in addition to the regular BD Rhapsody™ System workflow
- Stopping point for up to 24 hours during the workflow without compromising assay sensitivity

*WTA sensitivity means medium bioproduct/cell and molecule/cell. Based on development data using peripheral blood mononuclear cells (PBMCs)

One-stop-shop for IC CITE-seq experiments

BD® RNase Inhibitor

BD Rhapsody™ Intracellular AbSeq Buffer Kit

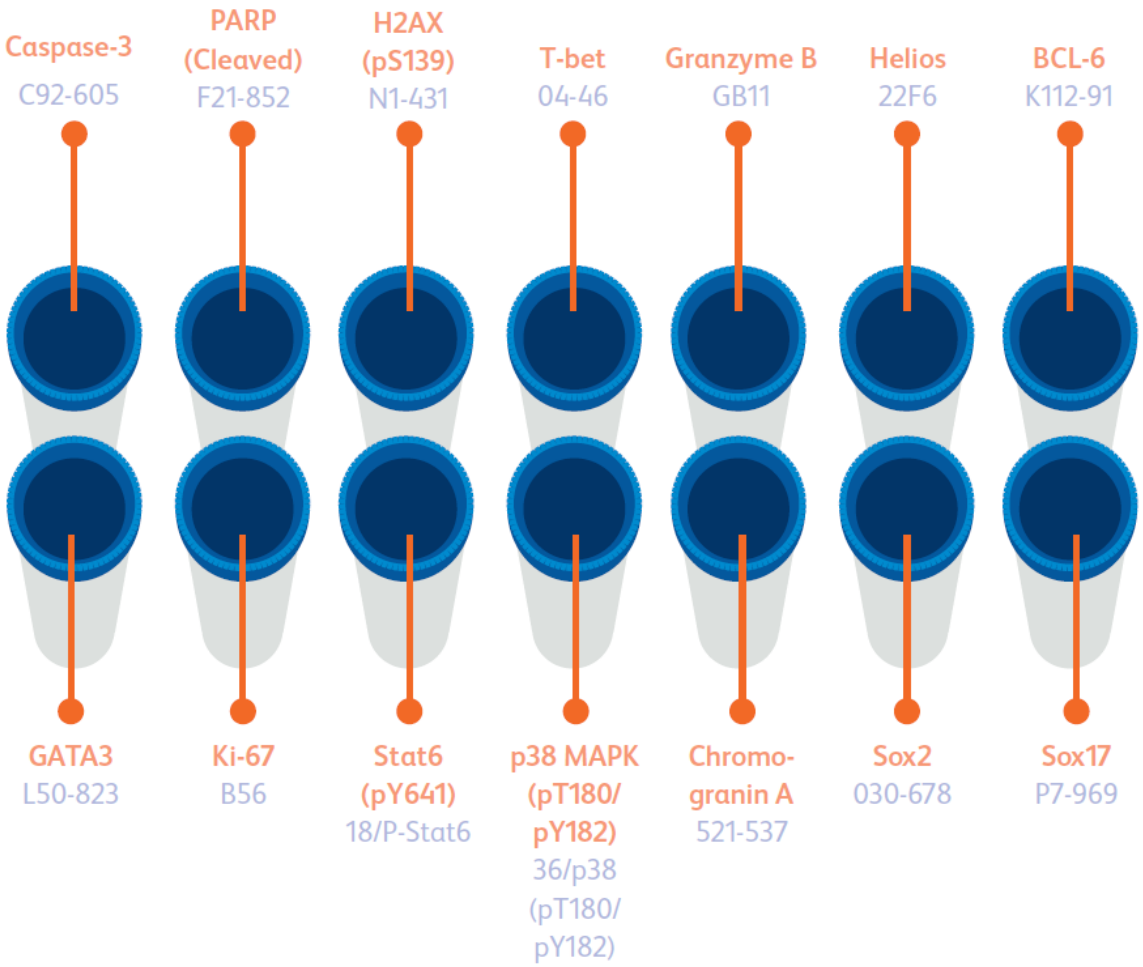
Intracellular BD® AbSeq Antibody-Oligos (14 specificities)

BD® OMICS-Guard Sample Preservation Buffer (2 sizes)

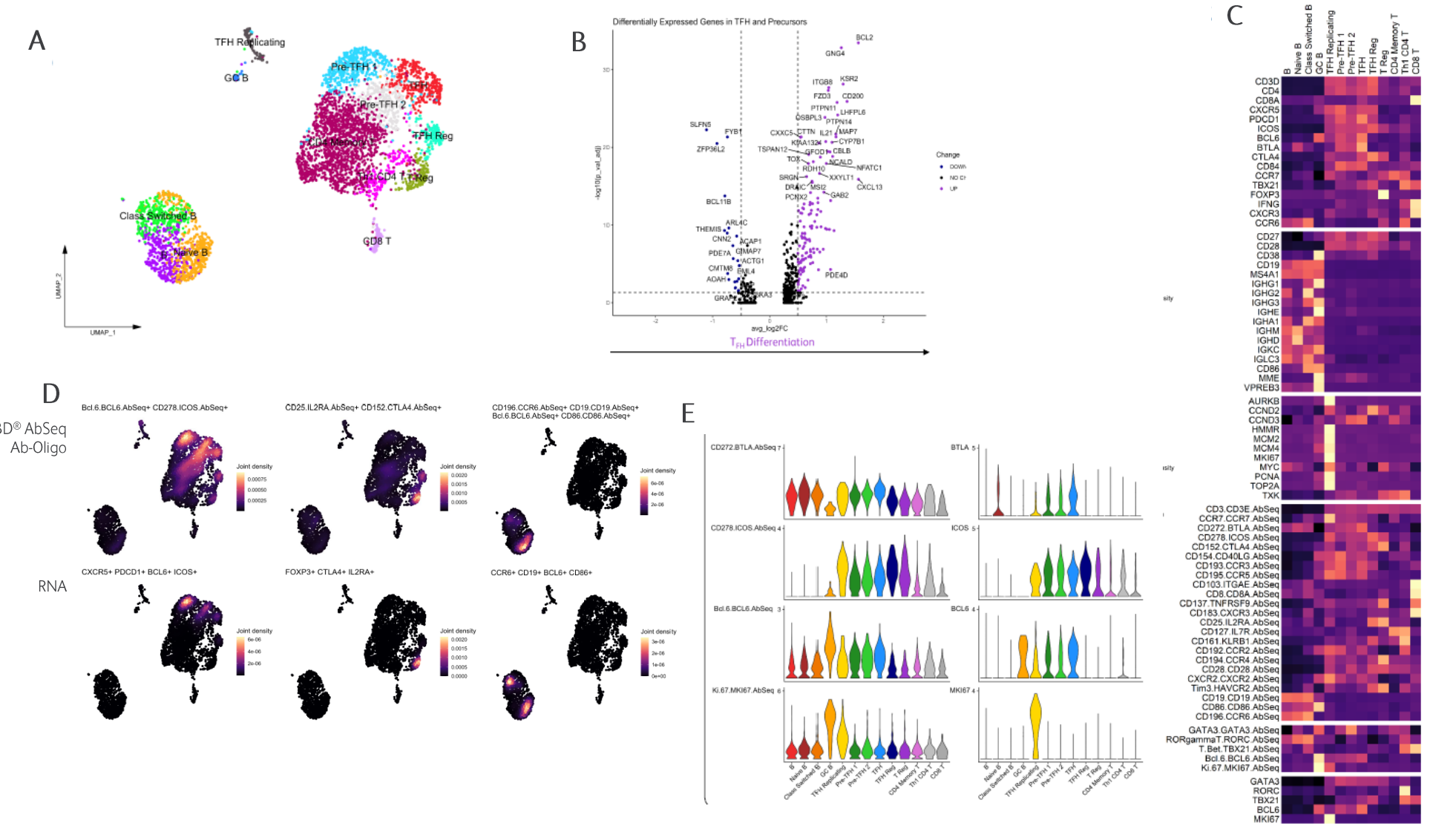
BD® AbSeq Enhancer Kit

Images not shown to scale.

Introducing intracellular BD® AbSeq Ab-Oligos for intracellular protein detection



A case study: Characterizing heterogeneous subset of T follicular helper (T_{FH}) cells in human tonsil with the Intracellular CITE-seq Assay using BD® AbSeq Ab-Oligos



Tonsillar T_{FH} cells were enriched and stained with both surface and intracellular BD® AbSeq Antibody-Oligos. **A)** Annotated UMAP of tonsil, colored by cell type. **B)** Statistically significant (Bonferroni adjusted *P* value (*P* val adj) > 0.05) versus fold change for differentially expressed genes (DEG) between T_{FH} cells compared to pre-T_{FH} cells. **C)** Heatmap visualizing the gene signatures and surface and intracellular BD® AbSeq Antibodies used to identify cell subsets within the tonsil. **D)** Top panels display surface and intracellular BD® AbSeq Antibodies used to identify T_{FH} cell (left), T regulatory cells (TReg) (middle) and B cells (right). Bottom panels display corresponding RNA signatures. **E)** Surface and intracellular BD® AbSeq Antibodies correlate with RNA expression within the tonsil.

Required reagents for the IC CITE-seq Assay

Required reagents for CITE-seq with BD® AbSeq Ab-Oligos

BD Rhapsody™ Enhanced Cartridge Reagent Kit

Cat. No. 664887, for four cartridge lane runs

BD Rhapsody™ Cartridge Kit

Cat. No. 633733, four cartridge lanes

OR

BD Rhapsody™ 8-Lane Cartridge

Cat. No. 666262, eight cartridge lanes

BD Rhapsody™ cDNA Kit

Cat. No. 633773, for four cartridge lane runs

BD Rhapsody™ WTA Amplification Kit

Cat. No. 633801, for four cartridge lane runs

Order the single-vial surface BD® AbSeq Ab-Oligos separately to suit experimental needs

+

Additional reagents needed for IC CITE-seq Assay

BD Rhapsody™ Intracellular AbSeq Buffer Kit

Cat. No. 570742, for four IC staining tests

- Base buffer, 20 mL
- Perm buffer, 12 mL
- 0.1 M DTT, 20 µL
- Proteinase K, 210 µL
- Nuclease-free water, 20 mL

BD® RNase Inhibitor

Cat. No. 570751, for four IC staining tests

Single-vial reagent, 900 µL

OR

RNase Inhibitor (New England Biolabs, M0314L)

Using RI from other vendors could impact assay performance

BD® AbSeq Enhancer

Cat. No. 570750, for four IC staining tests*

- BD® AbSeq Enhancer 1, 50 µL
- BD® AbSeq Enhancer 2, 50 µL
- BD® AbSeq Enhancer 3, 50 µL

BD® OMICS-Guard Sample Preservation Buffer Kit

Cat. No. 570908, for 12 million cells

12 vials/kit, 1 test/vial (1 mL)

OR

BD® OMICS-Guard Sample Preservation Buffer

Cat. No. 570911

50-mL bottle

Need to aliquot in sterile conditions

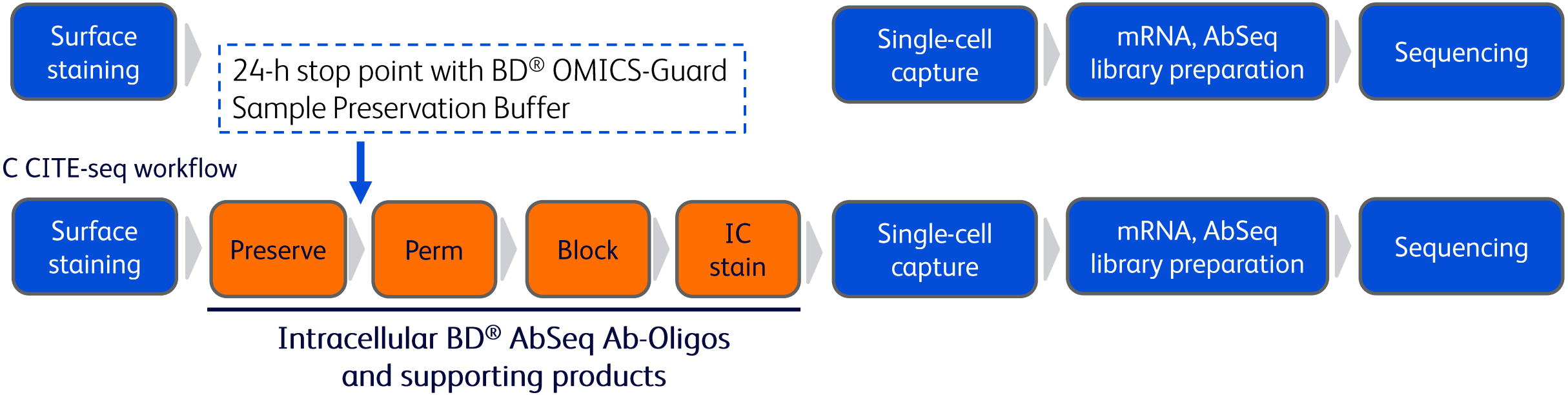
Vybrant™ DyeCycle™ Green Stain (Thermo Fisher Scientific, V35004)

Order the single-vial IC BD® AbSeq Ab-Oligos separately to suit experimental needs

*Optimization of the AbSeq Enhancer may be necessary for untested model systems to optimize signal to noise.

IC CITE-seq Assay overview

Surface CITE-seq workflow

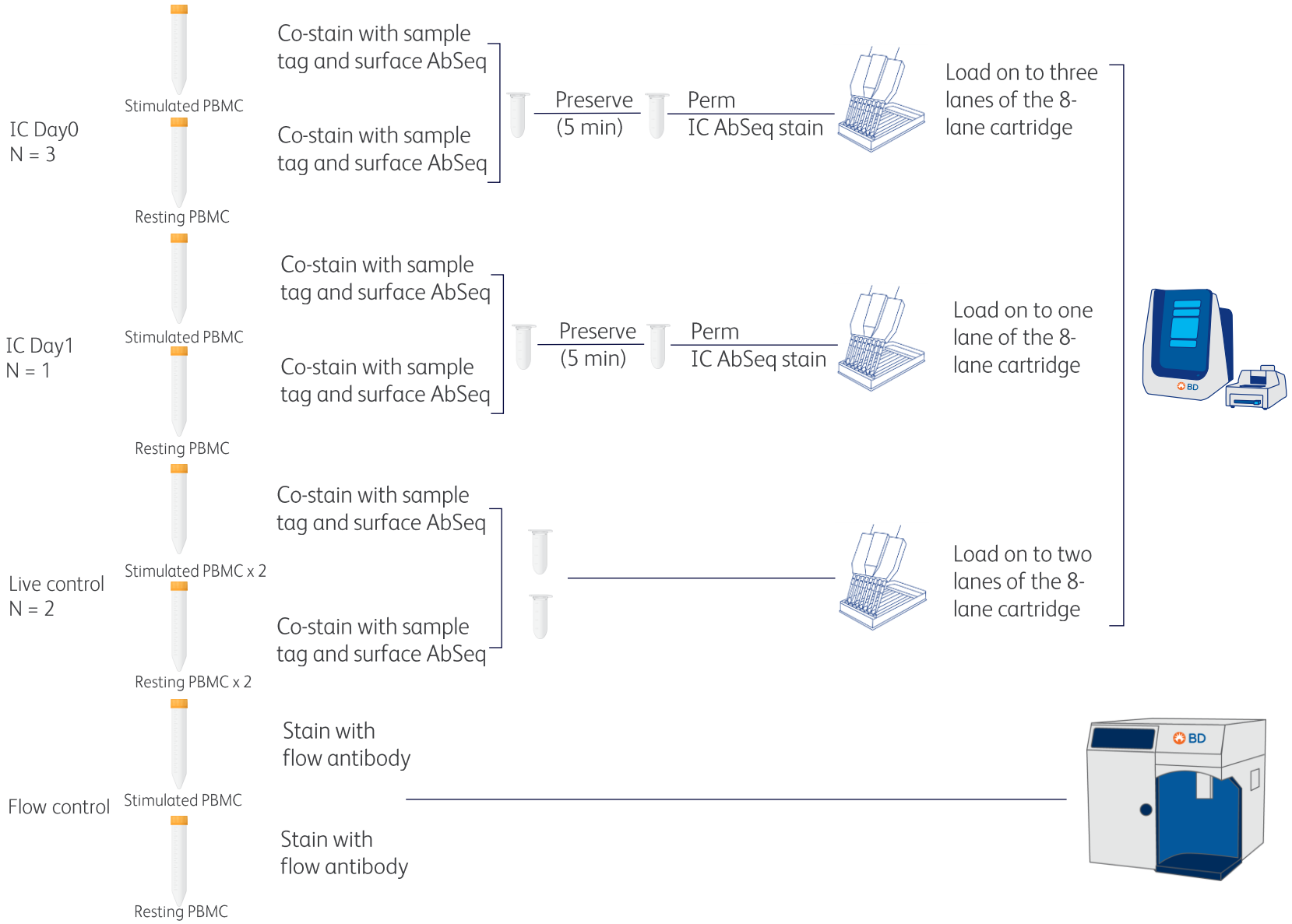


- **Preserve:** Preserves cell status
  - **Permeabilization:** Allows entry of AbSeq molecule into cell
    - **Blocking:** Limits noise from nonspecific binding of AbSeq Ab-oligos
      - **IC staining:** IC AbSeq Ab-oligos bind to intracellular proteins

Assay performance

Data from a CITE-seq study using previously frozen human PBMCs and a 49-plex BD® AbSeq Panel including nine IC markers

Experimental setup



Stimulation treatment

- PMA and ionomycin (calcium ionophore) 48-hour stimulation

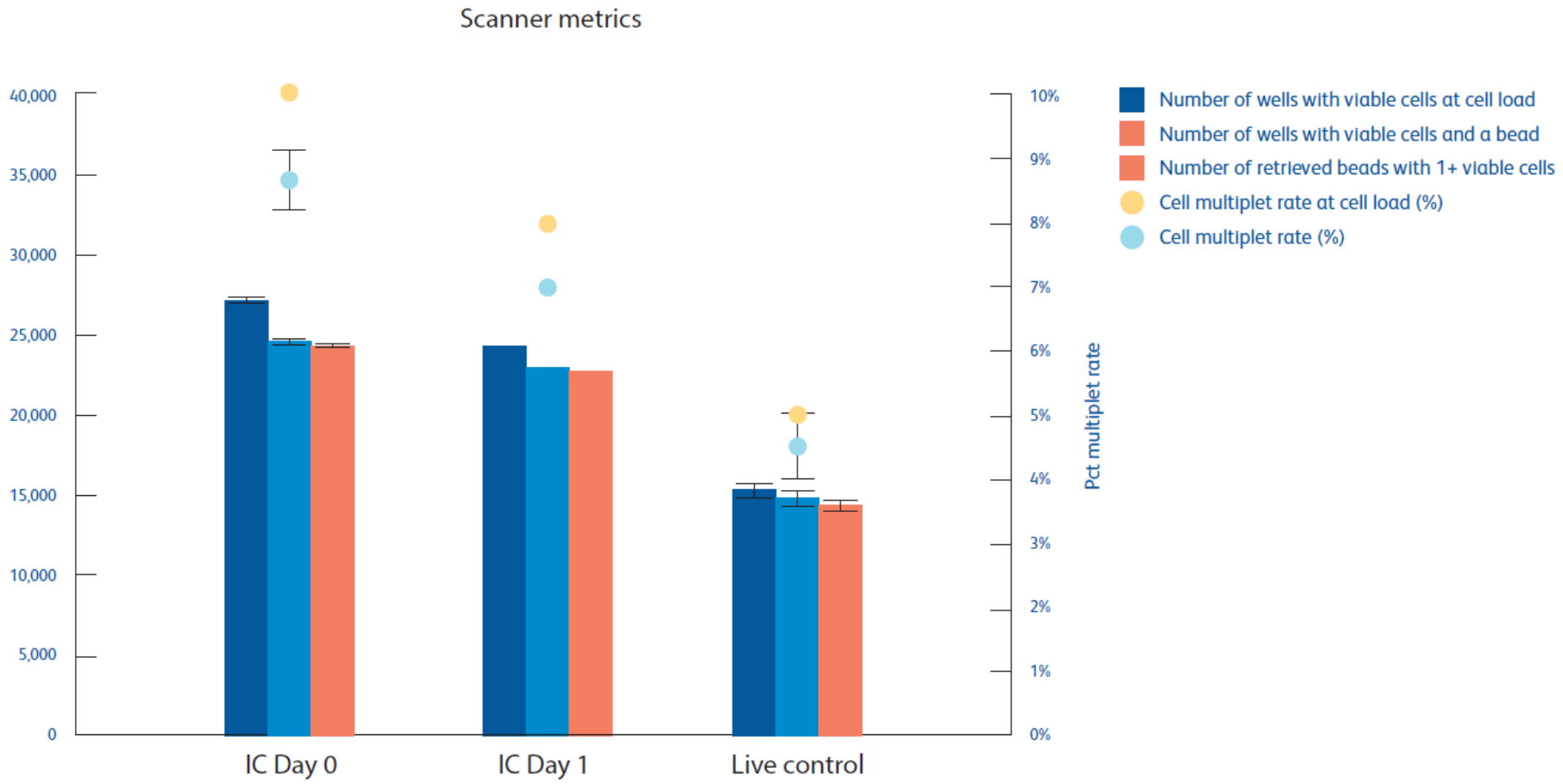
BD® AbSeq Panel

- 40 surface + 9 IC BD® AbSeq Ab-Oligos

IC specificity	Clone	Note
Do not expect expression		
GATA-3	L50-823	Not expected in PBMCs
BCL-6	K112-91	Not expected in PBMCs
Do not require stimulation		
Helios	22F6	Expressed in T cell subset
T-bet	O4-46	Expressed in lymphocyte subset
Granzyme B	GB11	Expressed in T cell subset
Require stimulation		
PARP (Cleaved)	F21-852	Upregulated in treated
H2AX (pS139)	N1-431	Upregulated in treated
Caspase 3	C92-605.rMab	Upregulated in treated
Ki-67	B56	Upregulated in treated

Cat. no.	Description	Clone
625970	BD® AbSeq Immune Discovery Panel	NA
940074	TCR-alpha_beta	TRA_TRB
940053	CD154	CD40LG
940049	CD40	CD40
940016	CD20	2H7
940029	CD7	CD7
940046	CD20	CD2
940019	CD69	CD69
940020	CD123	IL3RA
940023	CD64:10.1	FCGR1A
940037	CD95	FAS

Low multiplet rate at cell loading

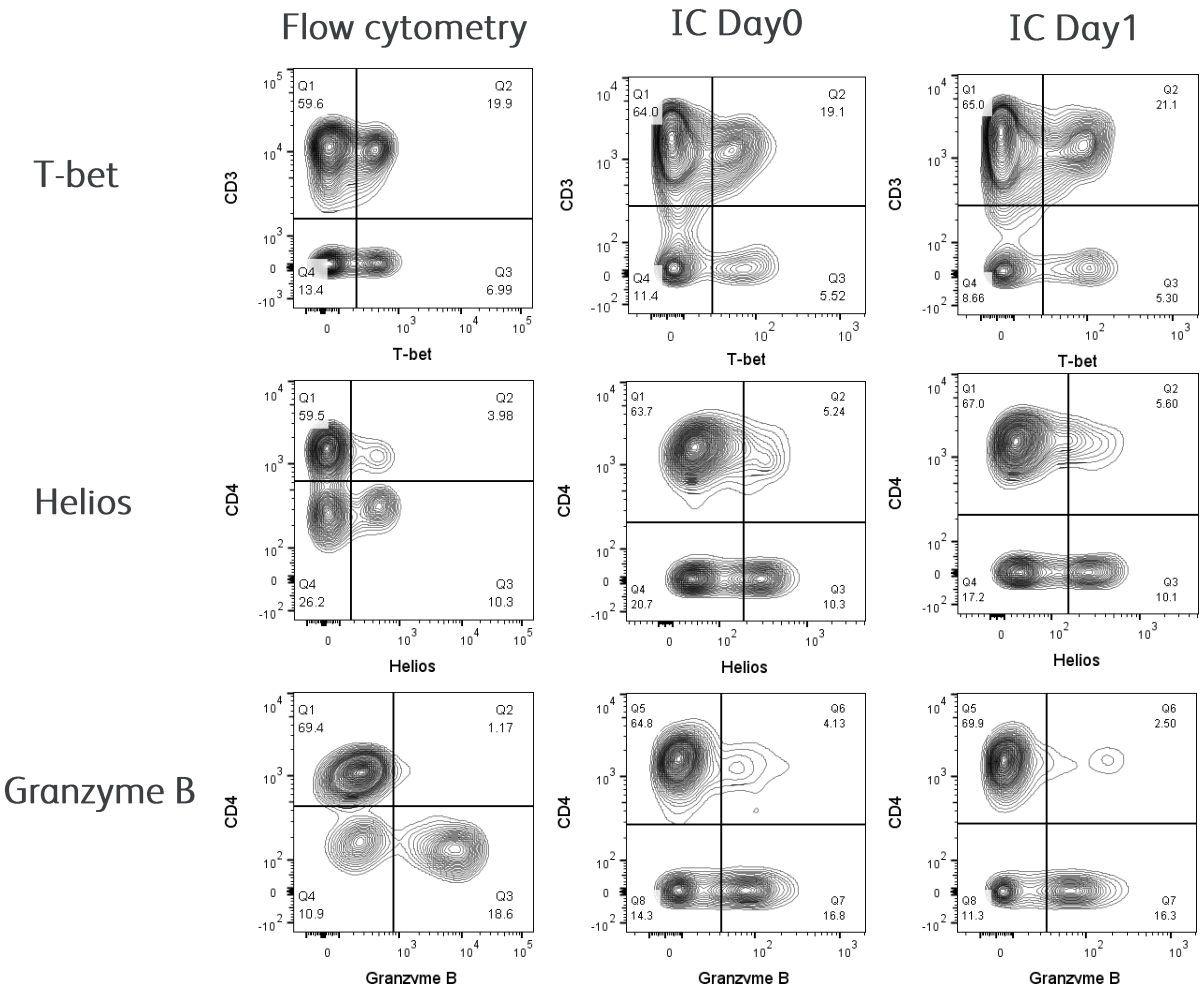


The IC Day1 sample was loaded on three lanes, IC 24-h was loaded on one lane, and live control samples were loaded on two lanes of the 8-lane cartridge on the BD Rhapsody™ HT Xpress System. The BD Rhapsody™ Scanner was used to monitor cell loading metrics, and low multiplet rate (<10%) was observed at ~26,000 cell loading.

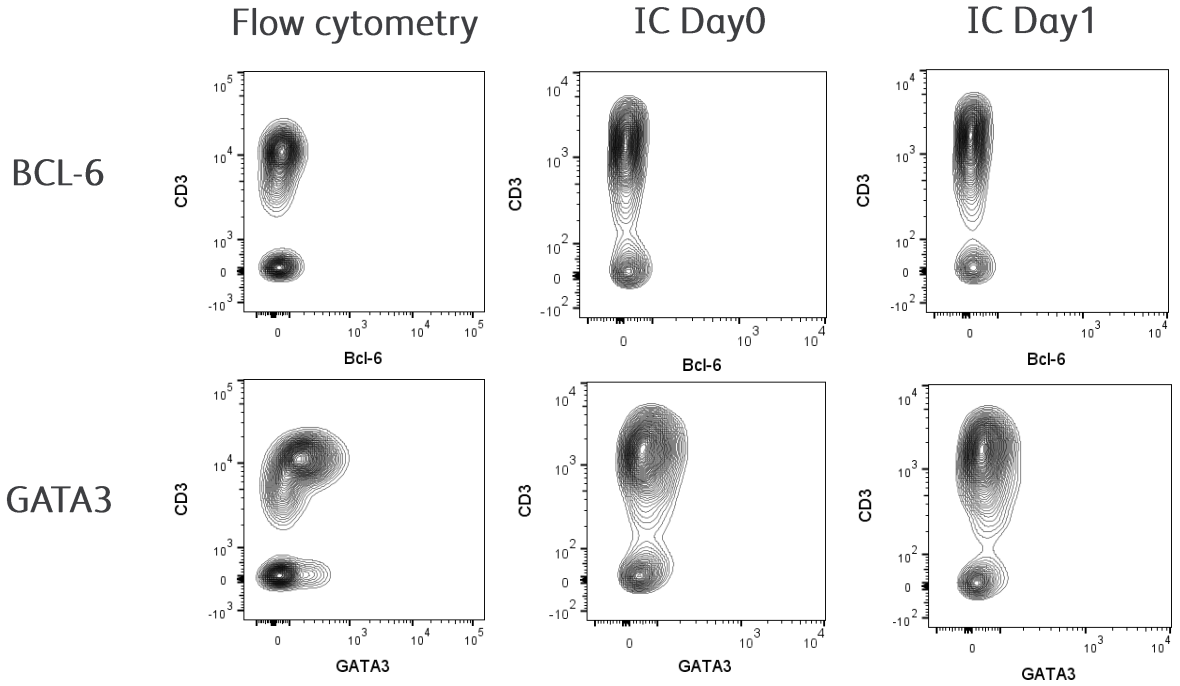
IC samples had cell multiplet rate <10% at cell loading with ~26,000 cells, suggesting IC CITE-seq workflow with BD® AbSeq Ab-Oligos does not significantly impact single-cell suspension.

High concordance between intracellular protein expression detected with intracellular BD® AbSeq Ab-Oligos and flow cytometry

T-bet, Helios and Granzyme B are expected to express in resting PBMCs



BCL-6 and GATA3 are not expected to express in PBMCs



Contour plots show expression of each IC protein in corresponding subsets of resting PBMCs. The IC protein expression is detected by flow cytometry and IC CITE-seq using BD® AbSeq Ab-Oligos (IC Day0 and IC Day1). Intracellular BD® AbSeq Ab-Oligos show consistent performance between 5-min and 24-h preservation workflows and show expected performance compared to flow cytometry controls.

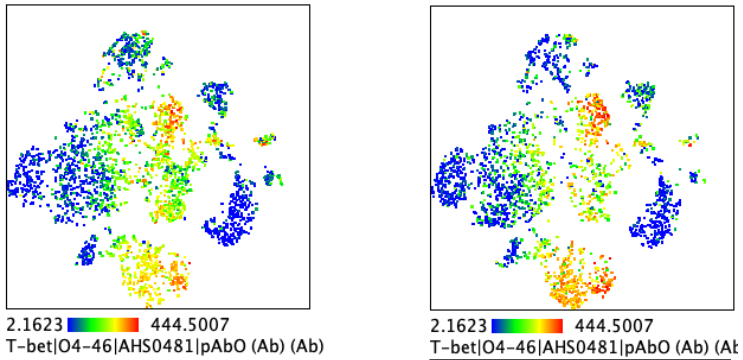
Optimization of the AbSeq Enhancer may be necessary for untested model systems to optimize signal to noise.

High concordance between intracellular protein expression detected with intracellular BD® AbSeq Ab-Oligos and flow cytometry

IC Day0 | IC Day1

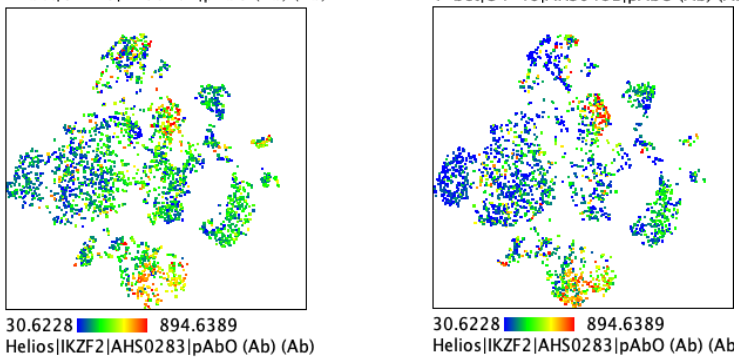
T-bet

Expression on PBMC lymphocyte subset



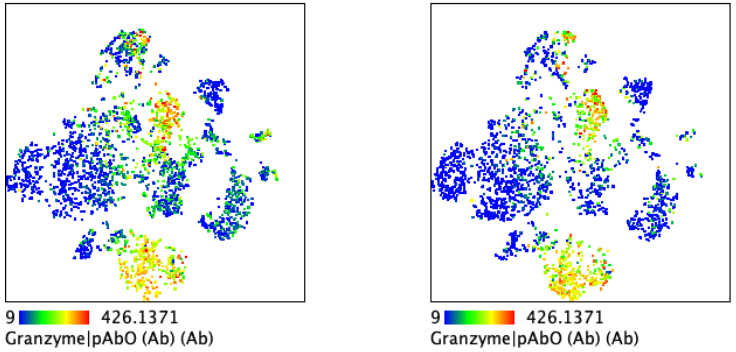
Helios

Expression in PBMC T cell subset

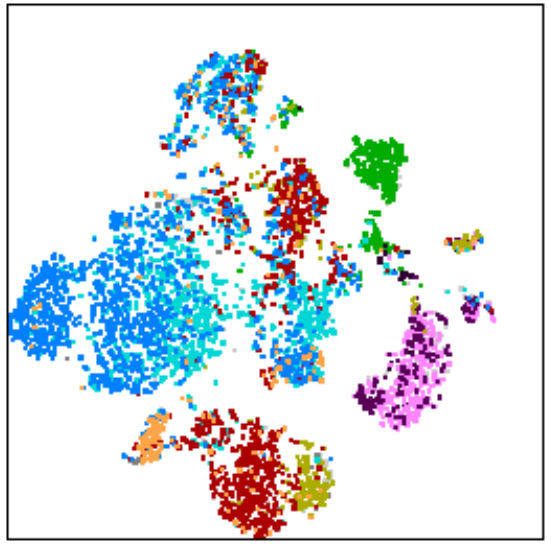


Granzyme B

Expression in PBMC T cell subset



Cell Type



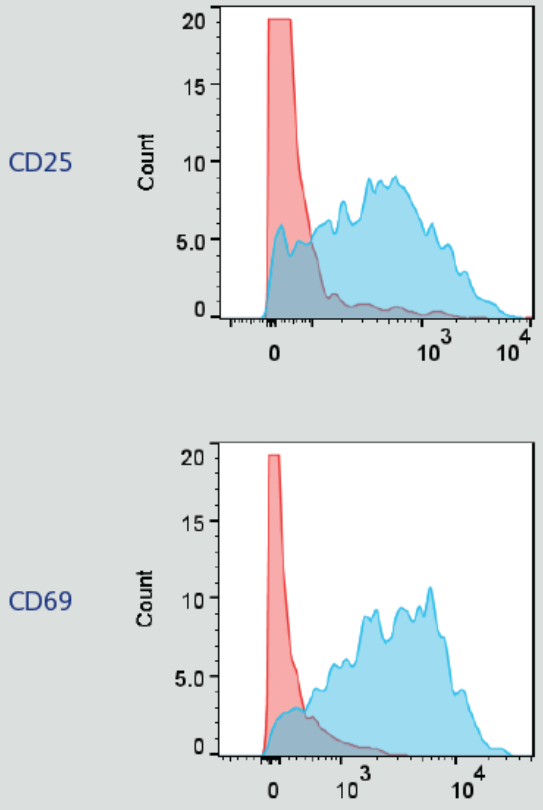
	Subset Name	Count
	T CD8 naive	227
	T CD4 naive	1462
	T CD8 memory	1133
	T CD4 memory	1614
	Natural killer cells	334
	Monocyte nonclassical cells	165
	Monocyte classical cells	473
	Gamma_delta cells	42.0
	Dendritic cells	64.0
	B cells	434
	Ungated	6276

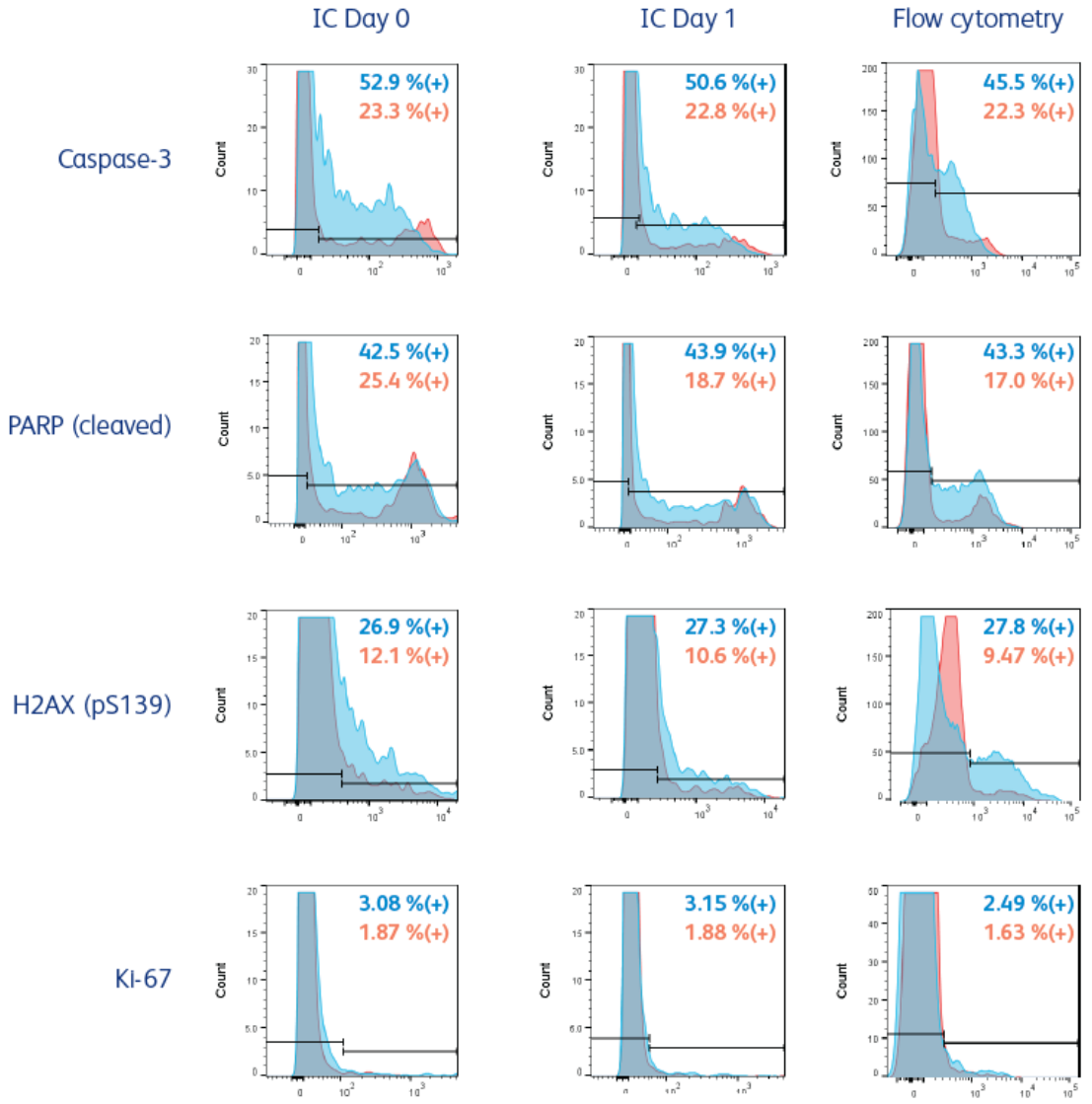
T-bet, Helios and Granzyme B expression is expected in subsets of resting PBMCs.

High concordance between intracellular protein expression detected with intracellular BD® AbSeq Ab-Oligos and flow cytometry

Surface markers (CD25 and CD69) are upregulated indicating stimulated phenotype

Treated with PMA and Ionomycin (Stimulated)
Untreated (resting)





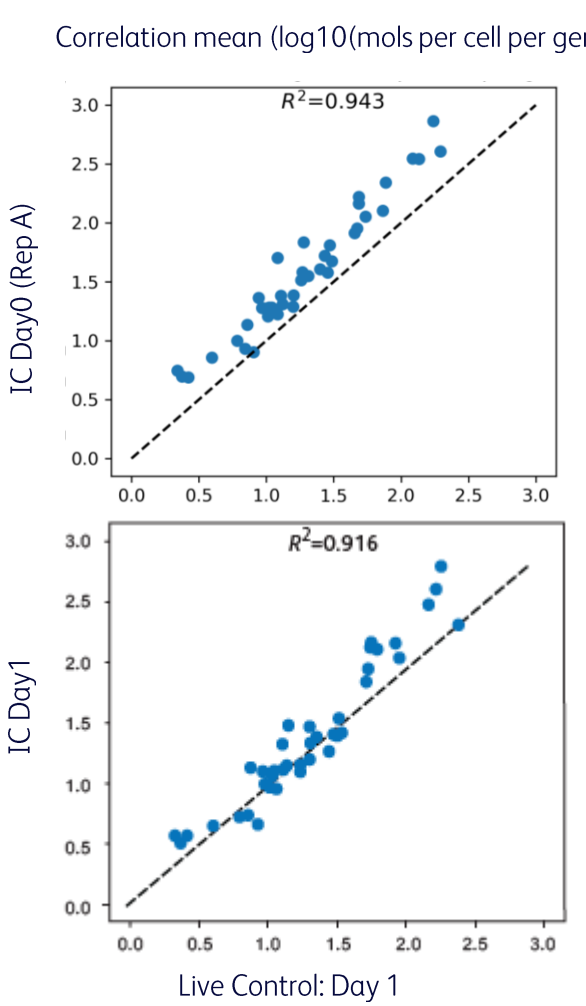
Histograms show BD® AbSeq Ab-Oligo detects similar percentage of induced apoptosis with stimulation as detected with flow cytometry

Histograms show BD® AbSeq Ab-Oligo detects similar percentage of proliferation with stimulation as detected with flow cytometry

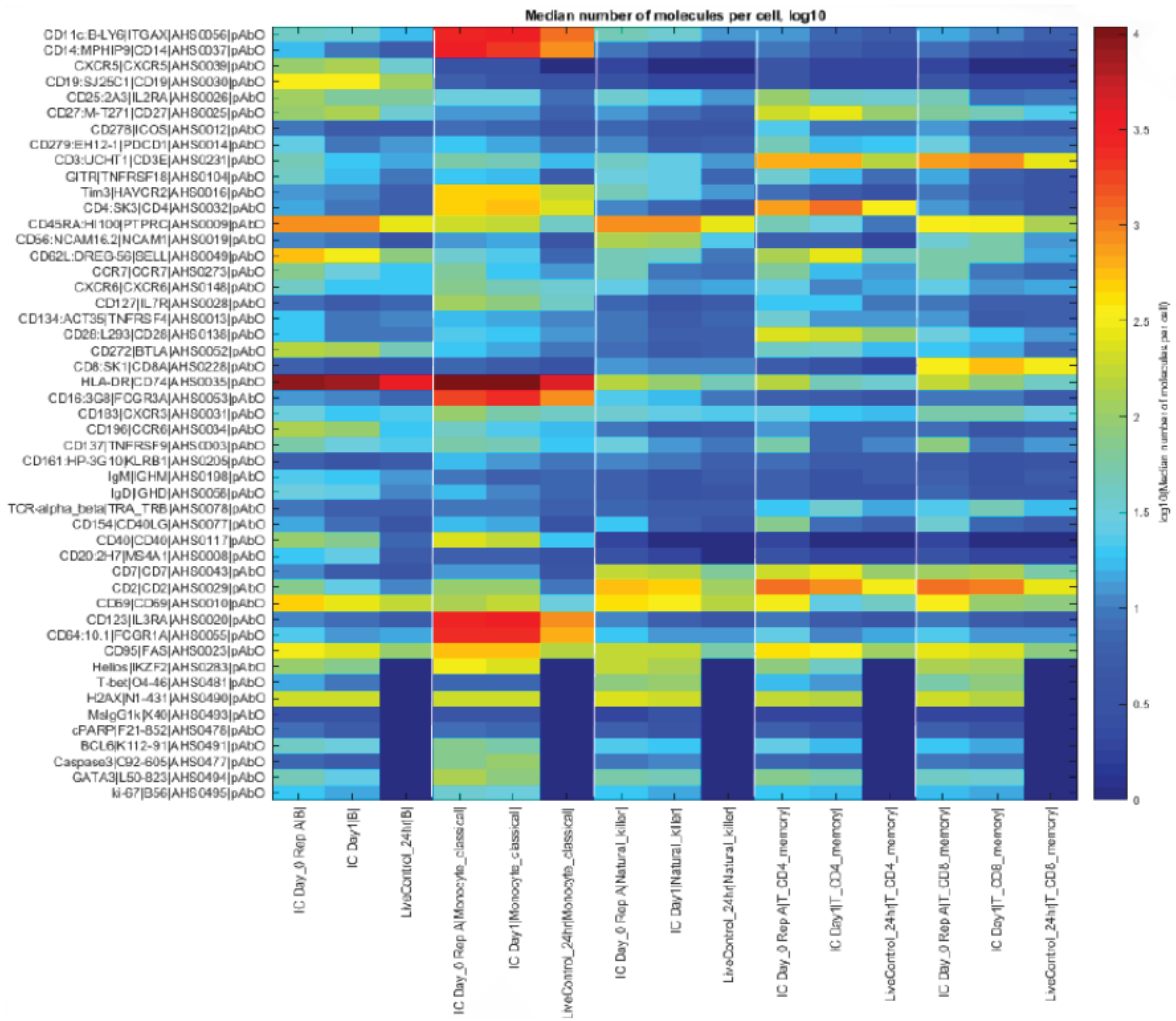
Optimization of the AbSeq Enhancer may be necessary for untested model systems to optimize signal to noise.

Profile surface proteins with high confidence while detecting intracellular proteins

A



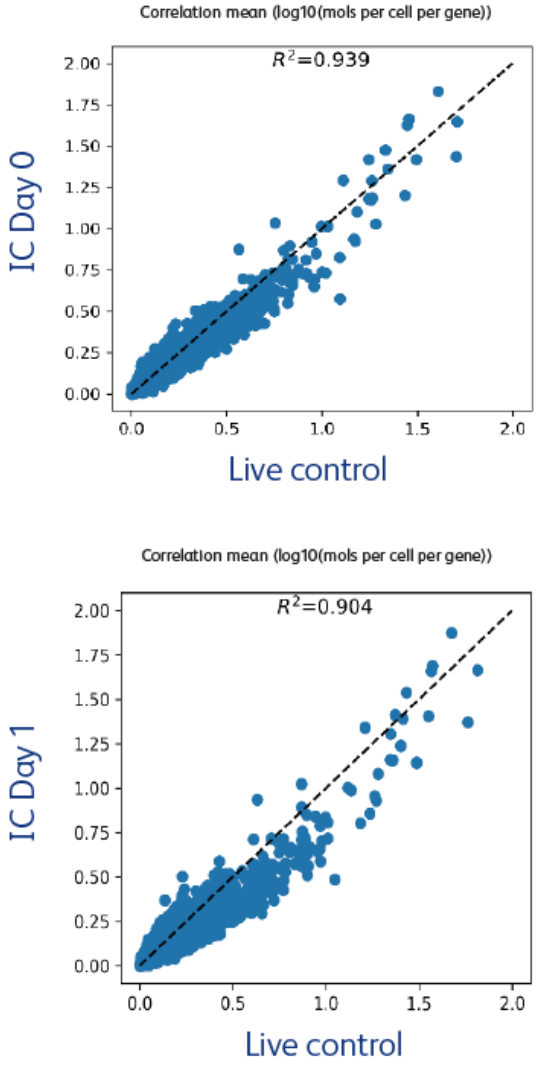
B



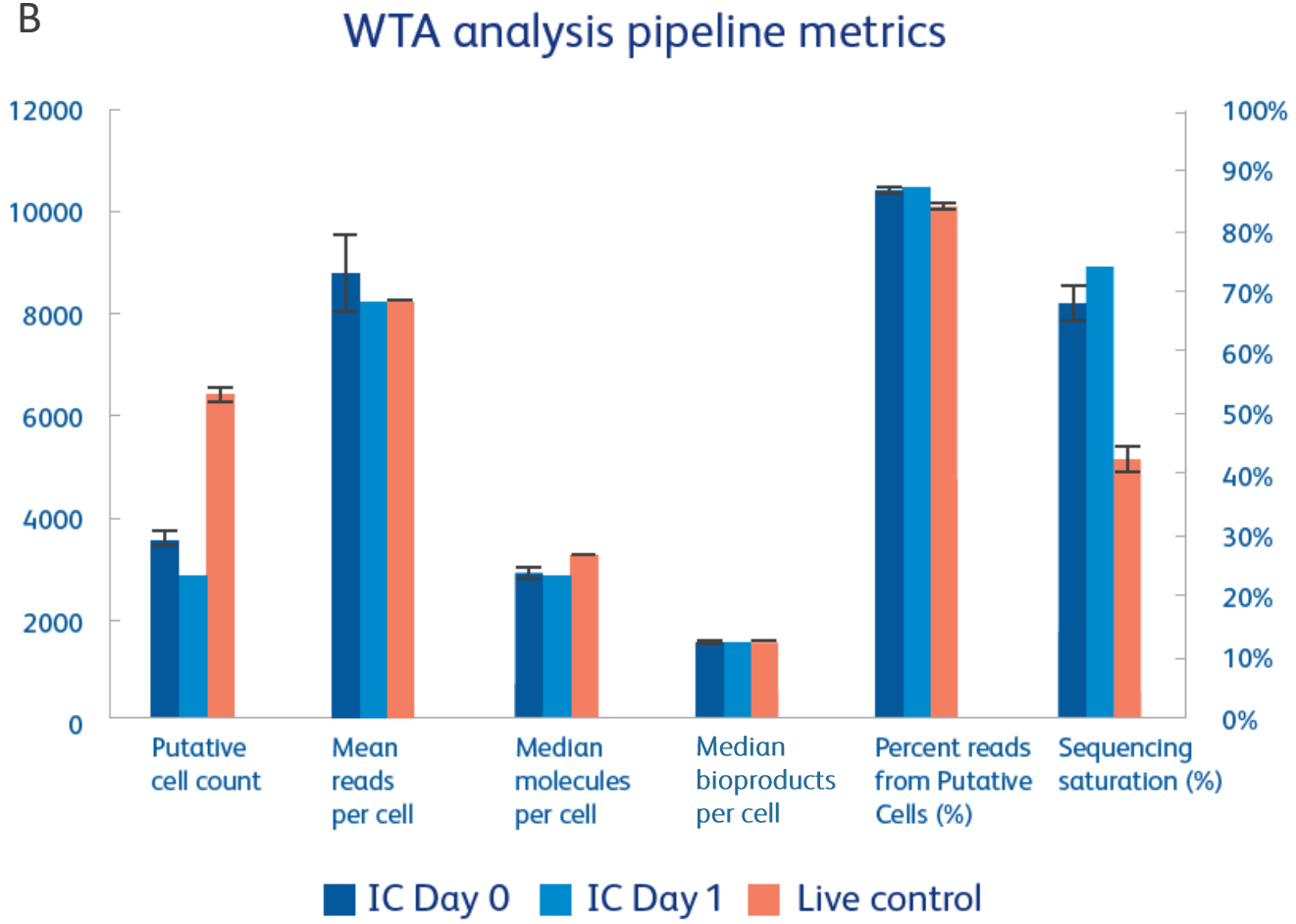
A) Surface AbSeq expression correlation between IC Day0 vs live control (top) and IC Day1 vs live control (bottom) are both R^2>0.9. B) Surface BD® AbSeq Ab-Oligo sensitivity represented by median molecules per cell of each BD® AbSeq Ab-Oligo in major PBMC cell types (B cells, classical monocytes, NK cells, CD4 and CD8 T cells). Cell type annotation is performed based on gene expression.

Reliable transcriptome analyses

A



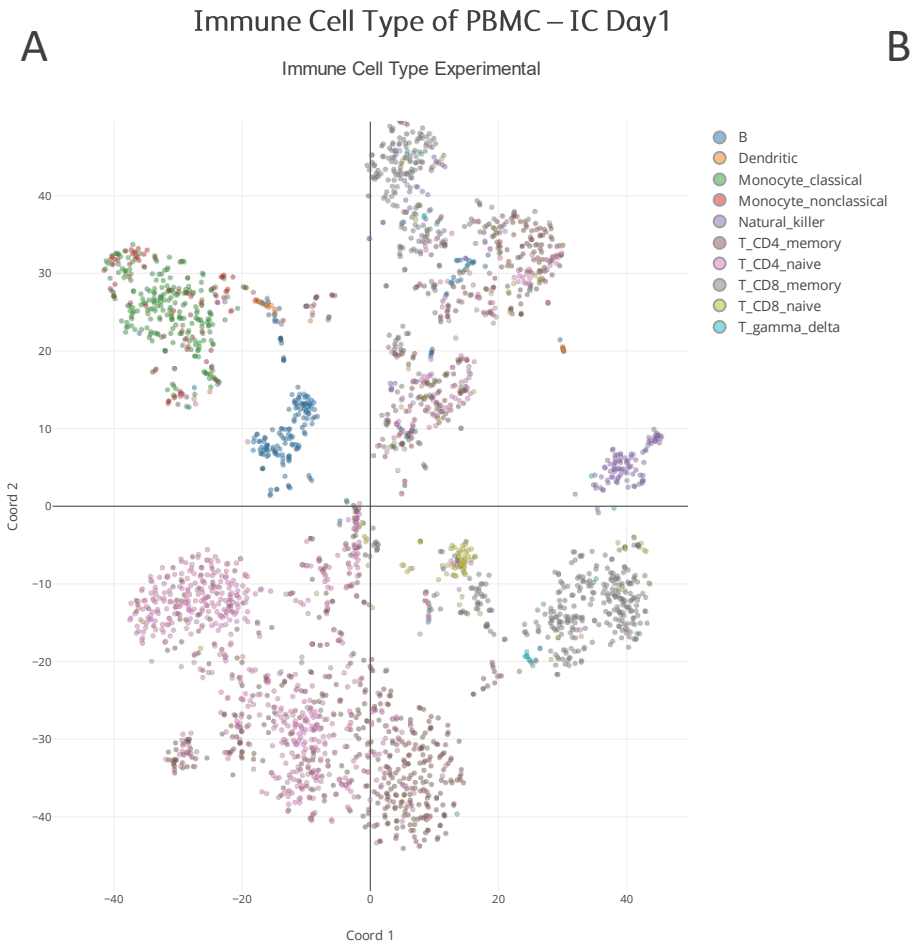
B



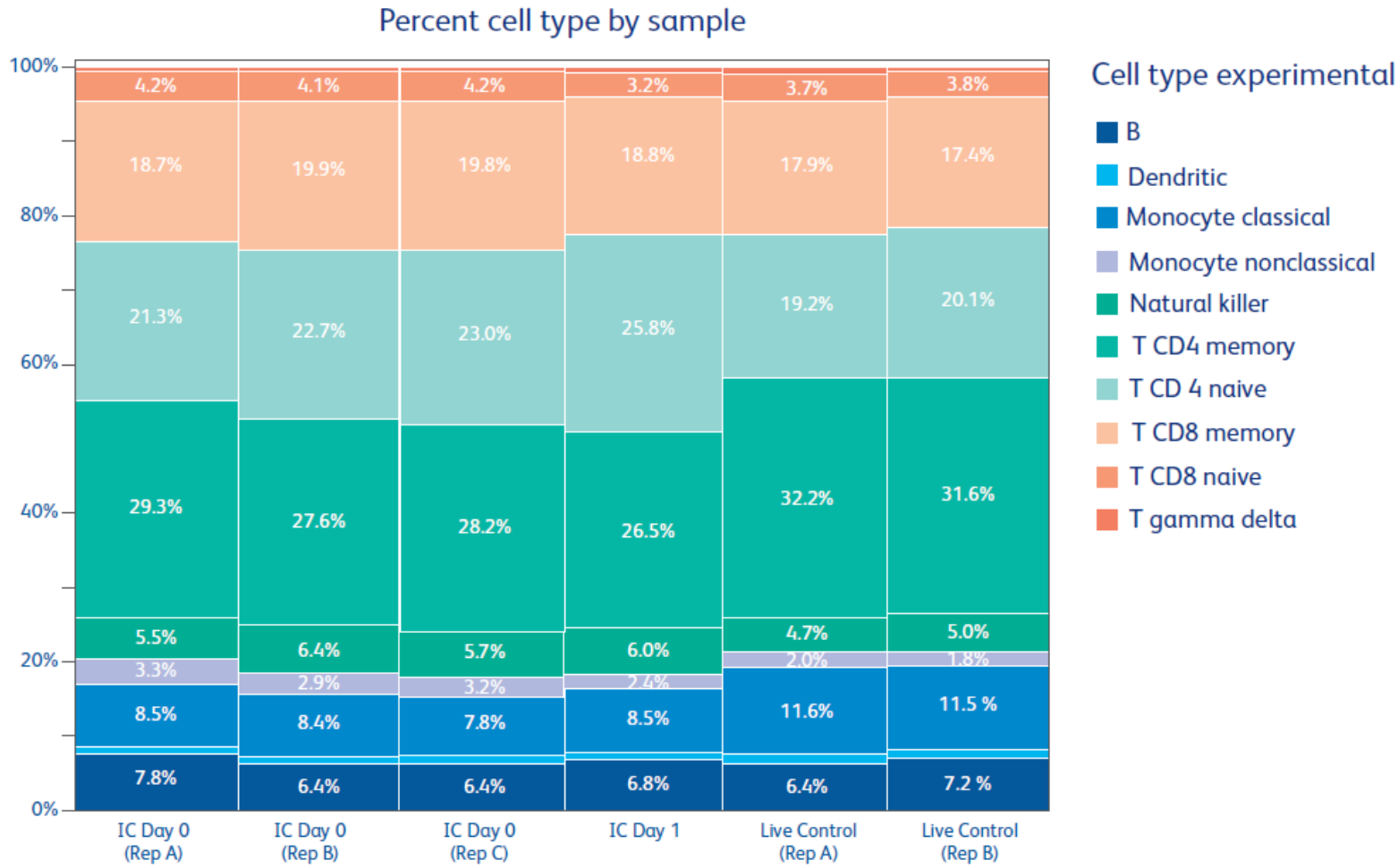
A) Gene expression correlation between IC Day0 vs live control (top) and IC Day1 vs live control (bottom) are both R^2>0.9. B) Samples were subsampled targeting 5,000 cells for sequencing. IC Day0 (n = 3) and IC Day1 samples (n = 1) recovered around 90% of WTA sensitivity compared to live controls (n = 2).

IC CITE-seq workflow using BD® AbSeq Ab-Oligos preserves major cell populations

A



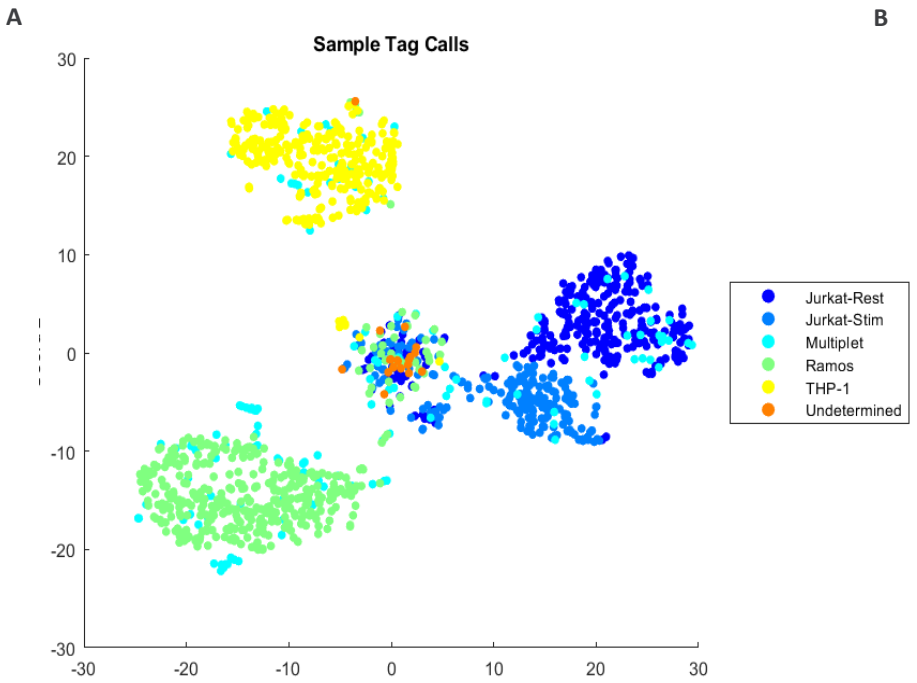
B

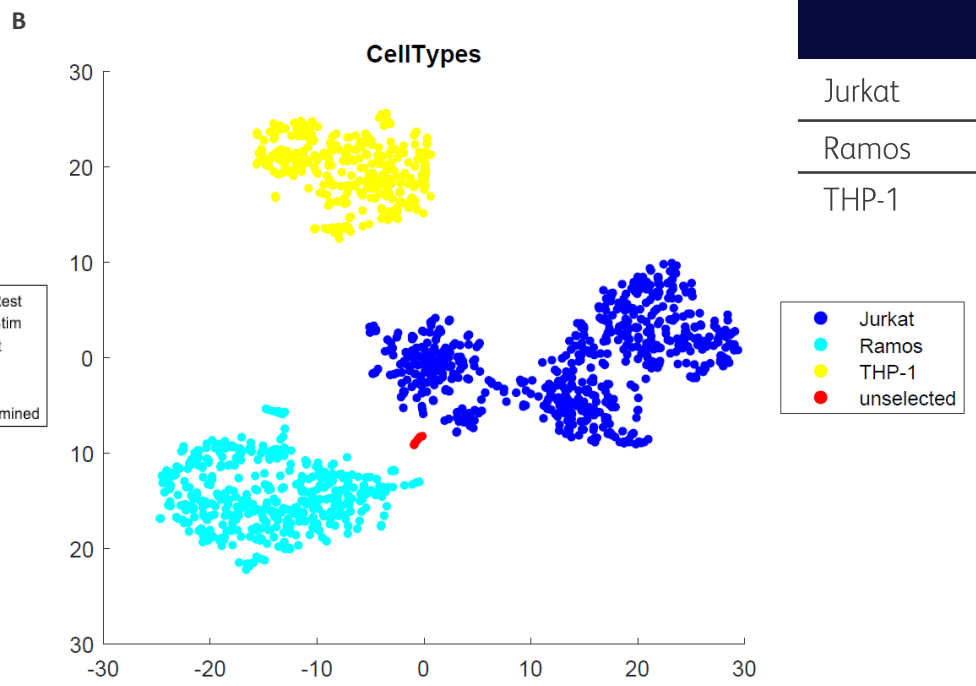


A) tSNE plot showing cell cluster and immune cell annotation in the IC Day1 PBMC sample. Immune cell classification is based on BD Rhapsody™ Sequence Analysis Pipeline Immune Cell Classifier. **B)** Major PBMC cell populations (B-cells, (non)classical monocytes, NK cells and T cells) were identified in IC Day 0 and IC Day1 samples, showing cell type frequency within 10% of total of live control

*Putative cells and cell clustering were based on mRNA-based cell calling algorithm

Sample multiplexing enabled





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

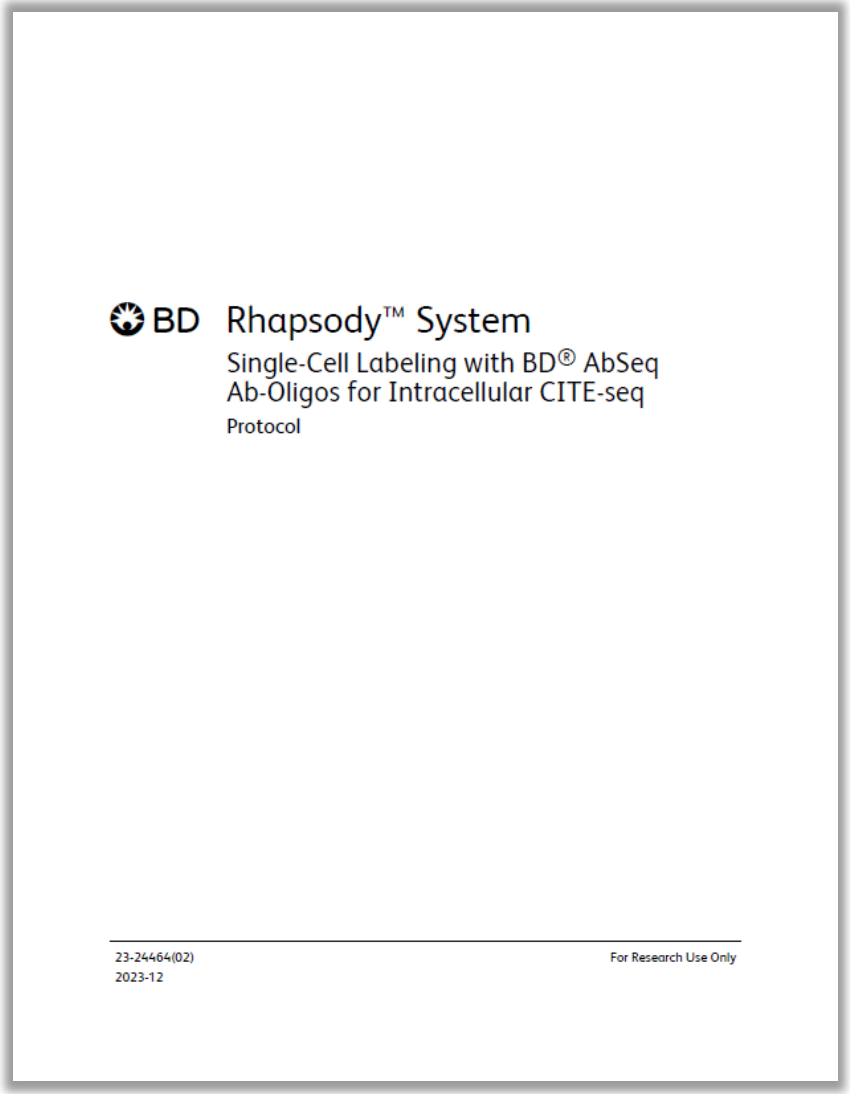
Sample multiplexing specificity and sensitivity for WTA + AbSeq + SMK are greater than 90%

Stimulated Jurkat treated with camptothecin and resting Jurkat, Ramos and THP-1 cell lines were co-stained with Human Sample Tag and IDP. The surface-stained samples were pooled and stained with IC BD® AbSeq Ab-Oligos (cPARP, BCL-6, Caspase-3, GATA3, H2AX) and analyzed with a WTA assay. For the purposes of calculating sample multiplexing specificity and sensitivity, Jurkat resting and stimulated were calculated as one cell line because they are difficult to differentiate in silico.

*SMK Sensitivity = 100% - Pct_of_Putative_Cells_Called (Undetermined)

*SMK Specificity = % Cell Count with expected Sample Tags among all cell types

Protocol, reagents and data analysis



Intracellular BD® AbSeq Ab-Oligo Staining Protocol

- BD Rhapsody™ Single-Cell Labeling with BD® AbSeq Ab-Oligos for Intracellular CITE-seq Protocol (23-24464)

Configuration and pricing (intracellular BD® AbSeq Ab-Oligos)

Cat. no.	Product description	Config (Size)	U.S. list price (USD)	Shelf life
940509	Helios Oligo AHS0283 22F6 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940510	Sox2 Oligo AHS0332 O30-678 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940511	Sox17 Oligo AHS0471 P7-969 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940512	Chromogranin A Oligo AHS0475 S21-537 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940513	Active Caspase-3 Oligo AHS0477 C92-605 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940514	PARP Oligo AHS0478 F21-852 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940515	T-bet Oligo AHS0481 O4-46 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940516	Stat6 Oligo AHS0482 18/P-STAT6 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940517	Granzyme B Oligo AHS0487 GB11 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940518	p38 MAPK Oligo AHS0489 36/P38 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940519	H2AX Oligo AHS0490 N1-431 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940520	BCL-6 Oligo AHS0491 K112-91 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940521	GATA3 Oligo AHS0494 L50-823 25Tst	25 tests/vial, 2 µL/test	$400	2 years
940522	Ki-67 Oligo AHS0495 B56 25Tst	25 tests/vial, 2 µL/test	$400	2 years

Configuration and pricing (associated reagents)

Cat. no.	Product description	Config (Size)	U.S. list price (USD)	Shelf life
570742	BD Rhapsody™ Intracellular AbSeq Buffer Kit	4 IC experiments	$250	2 years*
570750	BD® AbSeq Enhancer Kit	4 IC experiments	$500	2 years
570751	BD® RNase Inhibitor	4 IC experiments	$800	2 years
570908	BD® OMICS-Guard Sample Preservation Buffer Kit	12 tests, 1 mL/test	$195	2 years
570911	BD® OMICS-Guard Sample Preservation Buffer	50 mL	$225	2 years

Pricing current as of December 2023

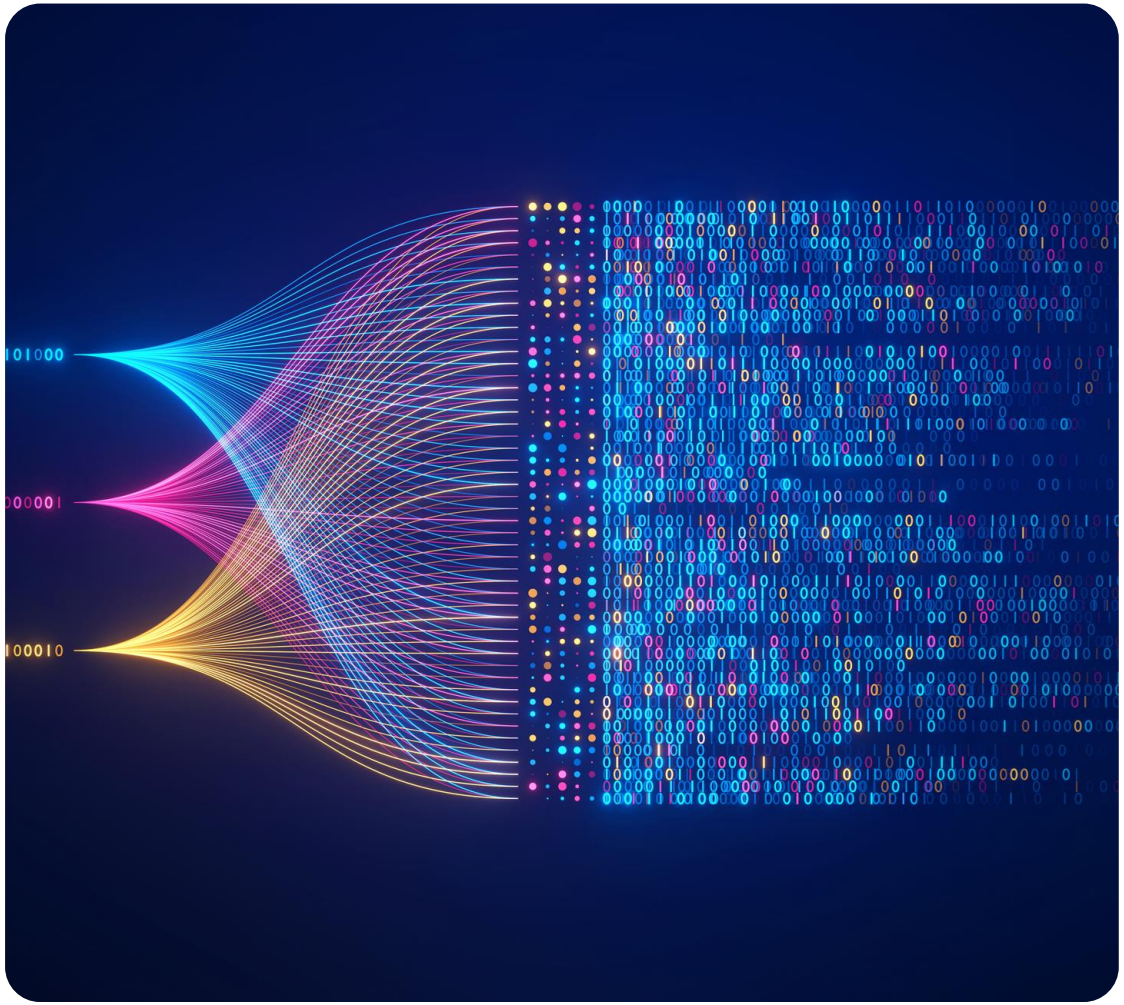
*May vary based on shelf life of kit components.

Required and suggested companion products

Cat. no.	Required companion product
633707 or 666625	BD Rhapsody™ Express Single-Cell Analysis System Package or BD Rhapsody™ HT Xpress System Package
633773	BD Rhapsody™ cDNA Kit
664887	BD Rhapsody™ Enhanced Cartridge Reagent Kit
633733 or 666262	BD Rhapsody™ Cartridge Kit or BD Rhapsody™ 8-Lane Cartridge
633801	BD Rhapsody™ WTA Amplification Kit
554656	BD Pharmingen™ Stain Buffer (FBS)

Cat. no.	Suggested companion product
633701	BD Rhapsody™ Scanner
Various	BD® AbSeq Ab-Oligos
633781	BD® Human Single-Cell Multiplexing Kit
625970	BD® AbSeq Immune Discovery Panel
564220	BD Pharmingen™ Human BD Fc Block™ Reagent

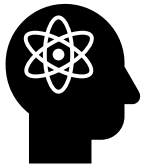
For a complete list of required and recommended materials, reagents, consumables and equipment, refer to the BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos for Intracellular CITE-seq Protocol (23-24464)



Sequencing data analysis using the BD Rhapsody™ Sequence Analysis Pipeline

- Get access to the BD Rhapsody™ Sequence Analysis Pipeline on the Seven Bridges Genomics Platform or on a local installation
- Acquire the AbSeq reference file (.fasta) from the BD AbSeq Panel Generator
- Set up analysis following the *BD® Single-Cell Multiomics Analysis Setup User Guide* (23-21333)

Supporting you with your single-cell experiments



Getting help from single-cell experts

Visit us at scomix.bd.com to view our resource library, learning center and FAQs

In need of technical support

BD technical service support is here to help with instrument support. Contact us **email** at scomix@bd.com or online at https://scomix.bd.com/hc/en-us/requests/new to submit a ticket

Ordering BD® AbSeq Ab-Oligos and intracellular CITE-seq products

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

Thank you