Providing total solutions to your immuno-oncology research workflow

Discover how BD can enable deep and robust data

BD

Table of contents

Supporting you in designing immuno-oncology assays from discovery research through clinical trials, BD is enabling scientific discoveries across various immuno-oncology research applications

Instrument platforms ▶ Reagents ▶ Immunology experts

At BD, we know that the goal of your research is to transform patient care by developing new cancer therapies. BD will support you in this noble journey by providing the high-quality tools to generate reliable data every time, which allows you the ability to advance immuno-oncology research.

Built on a solid foundation of over 40 years of experience in immunology, we bring unmatched legacy and expertise to help you achieve your goals and breakthrough new barriers in the field of immuno-oncology.

Whether you are studying new checkpoint inhibitors, evaluating multiple immunotherapy biomarkers, advancing CAR T-cell therapy research, developing new strategies for immune monitoring or discovering new frontiers in cancer vaccines—we're here to help simplify the complexity and guide you on your path from discovery research through clinical trials.

Visit **bd.com/Immuno-oncology** ◀

Increased performance for high-quality results: BD aims to provide a complete solution for your immuno-oncology research workflow

To have meaningful, profound effects on patient outcomes and quality of life, you need confidence in the data you generate. To move from early research through clinical trials and eventually to the clinic, you need reliable, optimized results with the ability to transfer high-quality data through each stage of your research.

Paving a path from discovery to clinical.
BD is dedicated to developing innovative, streamlined workflow solutions across the spectrum of discovery, translational and clinical research applications.

Bringing quality and reliability to high-complexity workflow solutions.
Our instruments, reagents and panels are seamlessly optimized, producing higher-quality data outputs.

Blood and tissue
collection and dissociation

Cell staining
antibodies, panels, dyes and reagents

Flow cytometry
cell sorters and cell analyzers

Multiomics
scRNAseq, AbSeq, gene panels and multiplexing

Informatics
flow and RNAseq analysis

Quality matters.
There's a patient at the end of everything we do.

Start with quality specimen collection to achieve better results

The BD Vacutainer® Products family: Cell and biomarker preservation

Global: Blood cell and biomarker preservation is widely used in clinical and biomarker research across the world

Flexible: Used with a variety of downstream molecular, multiomic and cellular applications

Reproducible: Helps ensure reproducibility and accuracy of data and workflow efficiency when measuring biomarkers

- Trusted by leading hospitals and research institutions to enhance sample quality, workflow efficiency and healthcare/laboratory personnel safety
- Backed by unrivaled customer support and training

BD Vacutainer® Products		
Cat no.	Description	Qty.
362753	BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube—sodium heparin	8.0 mL
362760	BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube—sodium citrate	4.0 mL
362761	BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube—sodium citrate	8.0 mL
7621165	PAXgene® Blood DNA Tube	2.5 mL
762165	PAXgene® Blood RNA Tube	2.5 mL

BD Horizon™ Dri Tumor and Tissue Dissociation Reagent: Gentle and effective dissociation with excellent epitope preservation

Gentle
Maximizes cell yields during dissociation while minimizing cell death

Effective
Efficiently dissociates a variety of tumor types to enable single-cell studies

Accurate
Maintains the heterogeneity and diversity of your samples

Figure A.

Marker	Pop	SI	MFI	% pos
CD1a				
CD1b				
CD1d	5			
CD2	4			
CD3	4			
CD4	4			
CD4v4	4			
CD5	4			
CD6	4			
CD7	4			
CD8a	4			
CD8b	4			
CD9	4			
CD10	1			
CD11a	4			
CD11b	6			
CD11c	6			
CD13	6			
CD14	5			
CD15	5			
CD15s	5			
CD16	6			
CD18	5			
CD19	3			
CD20	3			
CD21				
CD22				
CD23				
CD24	6			
CD25	4			
CD26	4			
CD27	4			
CD28	4			
CD29	4			

Marker	Pop	SI	MFI	% pos
CD30	4			
CD31	5			
CD32	6			
CD33	5			
CD34				
CD35	6			
CD36	5			
CD37	5			
CD38	5			
CD39	5			
CD40	1			
CD43	4			
CD44	6			
CD45	4			
CD45RA	3			
CD45RB	3			
CD45RO	6			
CD46	1			
CD47	1			
CD48	5			
CD49a	2			
CD49b	2			
CD49c	1			
CD49d	4			
CD49e	2			
CD50	6			
CD51/61	5			
CD53	6			
CD54	2			
CD55	6			
CD56	3			
CD57	3			
CD58	4			
CD59	1			

Marker	Pop	SI	MFI	% pos
CD61	5			
CD62E				
CD62L	4			
CD62P				
CD63	2			
CD64	5			
CD66 *(a-e)*	6			
CD66b	6			
CD66f				
CD69	5			
CD70	5			
CD71	1			
CD72				
CD73	2			
CD74	5			
CD75				
CD77				
CD79b				
CD80				
CD81	4			
CD83	5			
CD84	5			
CD85	5			
CD86	5			
CD87	6			
CD88	6			
CD89	5			
CD90				
CD91	5			
CDw93	6			
CD94	9			
CD95	5			
CD97	5			
CD98	5			

Tumor types evaluated by BD or external investigators include lung, breast, colon, lymphoma, melanoma/skin, pancreatic, esophageal, kidney, sarcoma and brain.

▶ Visit **bd.com/CDRR** or contact your local BD representative to learn how BD Horizon™ Dri Tumor and Tissue Dissociation can support your lab.

Excellent performance and preservation: 97% of the 188 cell surface markers were not compromised by the BD Horizon™ Dri Tumor and Dissociation Reagent

Marker	Pop	SI	MFI	% pos
CD99	5			
CD99R				
CD100				
CD102	5			
CD103				
CD105	1			
CD106				
CD107a	2			
CD107b	6			
CD108	5			
CD109	4			
CD112				
CD114	5			
CD116	5			
CD117				
CD118	2			
CD119	5			
CD120a	5			
CD121a	2			
CD121b	5			
CD122	3			
CD123	2			
CD124	5			
CD126				
CD127	4			
CD128b	5			
CD130	5			
CD134				
CD135				
CD137	5			
CD137L				
CD138				
CD140a	2			
CD140b	2			

Marker	Pop	SI	MFI	% pos
CD141	1			
CD142	1			
CD144				
CD146	2			
CD147	1			
CD150	4			
CD151	1			
CD152	2			
CD153	5			
CD154				
CD158a	3			
CD158b	9			
CD161	9			
CD162	5			
CD163	5			
CD164	5			
CD165	5			
CD166	5			
CD171	5			
CD172b	5			
CD177	7			
CD178				
CD180	5			
CD181	6			
CD183	4			
CD184	5			
CD193				
CD195	5			
CD196				
CD197				
CD200				
CD205	5			
CD206	5			
CD209	5			

Marker	Pop	SI	MFI	% pos
CD220	5			
CD221	1			
CD226	4			
CD227	5			
CD229	4			
CD231	1			
CD235a				
CD243				
CD244	5			
CD255	5			
CD268	3			
CD271	1			
CD273				
CD274	2			
CD275	5			
CD278				
CD279				
CD282	5			
CD305	5			
CD309				
CD314	4			
CD321	5			
CDw327	3			
CDw328	5			
CD329	5			
CD335	3			
CD336				
CD337	3			
CD338	5			
CD340	1			
abTCR	4			
b2-microglobulin	4			
BLTR-1	6			
CLIP	3			

Marker	Pop	SI	MFI	% pos
EGF Receptor	1			
fMLP receptor	5			
gdTCR	4			
HPC				
HLA-A,B,C	5			
HLA-A2	5			
HLA-DQ	5			
HLA-DR	5			
HLA-DR, DP, DQ	5			
Invariant NK T				
DGD2	5			
MIC A/B	5			
NKB1				
SSEA-1	6			
SSEA-4	2			
TRA-1-60	3			
TRA-1-81				
Vb 23				
Vb 8				
CD326	1			
CD49f	1			
CD104	1			
CD120b	5			
CD132	5			
CD201	1			
CD210	5			
CD212				
CD267	5			
CD294	6			
CLA	6			
Integrin b7	10			

Figure B.

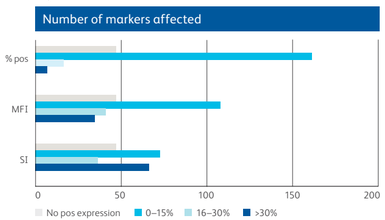


Figure 1. Preservation of cell-surface epitopes.
(A) Surface marker expression was assessed across the BD catalog for all 10 distinct cell population. Markers that demonstrated less than 20% expression are represented as grey boxes. For each marker, a single population *(T cells, non-T cells)* was chosen for the analysis. This target population is indicated by a number *(1–10) (column heading is "Pop," see poster abstract for population assignment numbers)*. Untreated and treated *(exposed to enzyme)* samples were compared using stain index (SI), mean fluorescence intensity (MFI) of positive signal and the % of the population that was clearly positive for a given marker *(% pos)*. Bright blue indicates a change of less than 15%, light blue indicates a change of 16%–30% and dark blue indicates a change of 30% or more.
(B) Graphical depiction demonstrating the majority of markers measured had preserved epitopes measured by MFI and SI, showing less than 0%–30% change following dissociation.

Middlebrook AJ, Austin C, Santos D, et al. The effects of enzymatic digestion on epitope detection by flow cytometry [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2018; April, 2018; Chicago, IL. Philadelphia, PA: AACR; Cancer Res. 2018;78(13 Suppl):Abstract nr 2113.

Ensuring precious samples are preserved right the first time.

BD Horizon Brilliant™ Dyes and Antibodies—color your world: Discover how these innovative dyes can give you confidence in your results with improved resolution and flexibility in experimental design

Our comprehensive portfolio of over 9,000 immunology and immuno-oncology related reagents are designed to help efficiently identify key insights—faster—by enabling characterization of the cells and biology relevant to your research

Brighter: For rare cells like tumor-infiltrating lymphocytes, or cells that have few receptors on the surface, bright reagents are essential in distinguishing these dim cells from others in a sample

Pioneering: BD Horizon Brilliant™ Polymer Dyes were developed from advanced Sirigen dye technology, enabling higher parameter flow cytometry experiments in order to better discern populations and garner deeper insights

Reliable: Lot-to-lot consistency and a commitment to high quality ensures better performance and confidence throughout longitudinal studies

Breaking barriers to discovery starts with the right reagents and panels. BD has an expansive portfolio of dyes across multiple laser lines with a multitude of antibody conjugates for each dye—providing you with the choice and flexibility you need to take your research to the next level.

BD OptiBuild™ Reagents provides on-demand access to thousands of antibody-dye conjugate combinations to enable flexible panel design

Expand your panel design possibilities with BD OptiBuild™ On-Demand Reagents by uncovering more fluorochrome options on the antibodies you need

Simple and flexible: Whether you want to minimize compensation or add new markers to complex experiments, BD OptiBuild™ Reagents provide flexibility to evaluate new colors and simplify your panel design

- Access to new antibody-dye combinations made on demand with rapid turnaround times to help you meet research deadlines
- Consistent performance to ensure reliable results

Fast and convenient: Unlike traditional large-scale, expensive custom conjugates, these reagents:

- Come in convenient 50-µg vials
- Can be ordered as a catalog reagent for optimal panel design
- Are made on demand, and usually ship in less than 72 hours*

*Within U.S., Canada and Europe

Visit **bd.com/Optibuild**

Predesigned BD Horizon™ Dri Panels: Dried reagent cocktails for improved workflow efficiency and consistency

Minimize time spent on cocktailing reagents and reduce day-to-day variability

BD offers predesigned *(consensus)*, performance-optimized panels in dried down, ready-to-use multicolor panels optimized and tested for memory T-cell, monocyte subset and TBNK cell characterization.

Easy-to-use*: All-in-one, multicolor panels optimized for cell characterization can be used across labs and sites to enable reliable results.

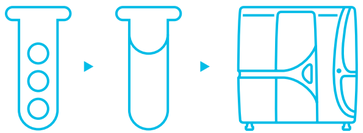
Lot-to-lot consistency*: High-quality reagents and BD technology built into a reagent panel. Long-term reagent stability further enhances assay reproducibility.

Stability*: Improved performance and shelf life over liquid cocktails with the advantage of room-temperature storage. Offers a performance guarantee for one year at room temperature.

Convenience*: Ready-to-use, dried-down, single-use tubes offering workflow efficiency while providing greater accessibility and convenience. Simply resuspend the dried reagents and add your sample, with no need for cocktailing.

*Versus liquid reagents

Improve ease-of-use and reduce time spent preparing reagent cocktails



BD Horizon™ Dri Panel Reagent | Add sample, vortex and incubate | Lyse, wash and analyze by flow cytometry

BD Horizon™ Dri Monoset Panel		
Fluorochrome	Marker	Clone
FITC	CD16	3G8
PE	HLA-DR	L243
PerCP	CD14	MφP9
APC	CD192 *(CCR2)*	LS132.1D9

BD Horizon™ Dri Memory T-Cell Panel		
Fluorochrome	Marker	Clone
FITC	CD197	150503
PE-Cy™7	CD95	DX2
BD Horizon™ APC-R700	CD27	M-T271
APC-H7	CD3	SK7
BD Horizon™ V450	CD4	SK3
BD Horizon™ V500-C	CD8	SK1
BD Horizon Brilliant™ Violet 605	CD45RA	HI100

BD Horizon™ Dri TBNK+CD20 Reagent Panel		
Fluorochrome	Marker	Clone
BD Horizon Brilliant™ Violet 450	CD20	L27
FITC	CD3	SK7
PE	CD16	B73.1
PE	CD56	NCAM16.2
PerCP-Cy™5.5	CD45	2D1 *(HLe-1)*
APC	CD19	SJ25C1
PE-Cy™7	CD4	SK3
APC-Cy™7	CD8	SK1

In addition to predesigned panels, BD Custom Technology Team (CTT) offers contract manufacturing of multicolor panels in lyophilized, liquid and/or dried formats to minimize the error(s) and time associated with manual cocktailing of reagents, increase the reagent stability, and significantly enhance performance consistency.

▶ Visit **bd.com/CDRR** and **bd.com/CustomPanels** or contact your local BD representative to learn how BD Horizon™ Dri Panels and custom panels can support your lab.

Regulatory T-cell panel: Identification of Treg subsets and understanding Treg heterogeneity on the BD FACSLyric™ Flow Cytometer

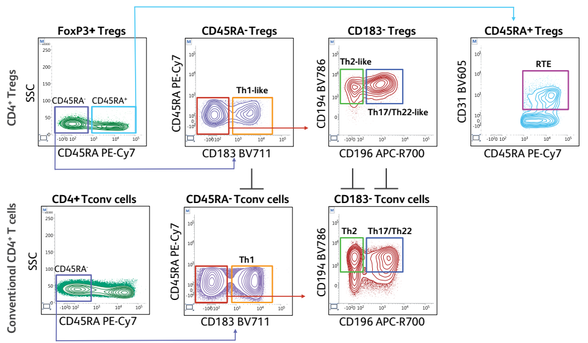


Figure 2. Demonstration of modular flow cytometry approach *(7 + 5 colors)* to characterize Tregs. The 7-color backbone panel was used to identify Tregs and was supplemented with a homing panel whose expression correlates with different aspects of Treg biology, such as homing, function, maturation, proliferation, trafficking and stability. The homing panel successfully identified 3 subpopulations of Th-like Tregs and recent thymic emigrants within the Treg population *(upper panel)*. Conventional T-helper cell subsets Th1, Th2 and Th17/Th22 were also identified within the conventional CD4+ T cells *(Tconv) (lower panel)*.

Corselli M, et al. A modular, multicolor approach to regulatory T-cell characterization. White paper. *BD Biosciences, San Diego, CA. 2018.*

Regulatory T-cell panel	
7-color Treg panel	Additional Treg homing drop-ins
CD127	FoxP3
CD15s	CD31
CD161	CD183
CD4	CD194
CD45RA	CD196
CD25	
CD3	

High-resolution identification of cell populations makes it an ideal solution for expanded immunophenotyping of immuno-oncology-relevant cell populations.

BD Horizon Brilliant™ Dyes and Antibodies are enabling discovery research for immunotherapy

Minimize your time spent troubleshooting and maximize your impact

Perform a comprehensive and detailed analysis of the immune system with optimized flow cytometry panels to determine which strains are a suitable host for patient-derived xenografts (PDX).

T-cell panel		
Cat no.	Markers	Fluorochrome/Dye
See mouse dump table below	Mouse dump	FITC
560662	CD8	PerCP-Cy™5.5
555480	CD38	PE
562381	CD197	PE-CF594
560684	CD28	PE Cy™7
555342	CD3	APC
560274	CD45	APC-H7
557922	CD4	AF700
562885	CD45RA	BV421
564406	Live/Dead cells	FVS 510
562846	HLA-DR	BV605
563159	CD25	BV711
563324	CD127	BV786
561908	DNA	Hoechst 33342

Myeloid panel		
Cat no.	Markers	Fluorochrome/Dye
See mouse dump table below	Mouse dump	FITC
560635	CD195	PerCP-Cy™5.5
560933	CD163	PE
564063	CD206	PE-CF594
557743	CD11b	PE Cy™7
561306	CD16	APC
560180	CD14	APC-H7
560566	CD45	AF700
564067	CD192	BV421
564406	Live/Dead cells	FVS 510
562845	HLA-DR	BV605
563171	CD33	BV711
563383	CD15	BV786
561908	DNA	Hoechst 33342

NK/DC/B-cell panel		
Cat no.	Markers	Fluorochrome/Dye
See mouse dump table below	Mouse dump	FITC
560835	CD3	PerCP-Cy™5.5
555413	CD19	PE
562393	CD11c	PE-CF594
562101	NKp46	PE Cy™7
561304	CD16	APC
560180	CD14	APC-H7
560566	CD45	AF700
562518	IgD	BV421
564406	Live/Dead cells	FVS 510
562845	HLA-DR	BV605
563161	CD123	BV711
564058	CD56	BV786
561908	DNA	Hoechst 33342

Mouse dump	
Cat no.	Antibody
553079	mCD45
553592	mH2Kd
561032	mTer119
558738	mCD31
561849	mCD41
553266	mCD71

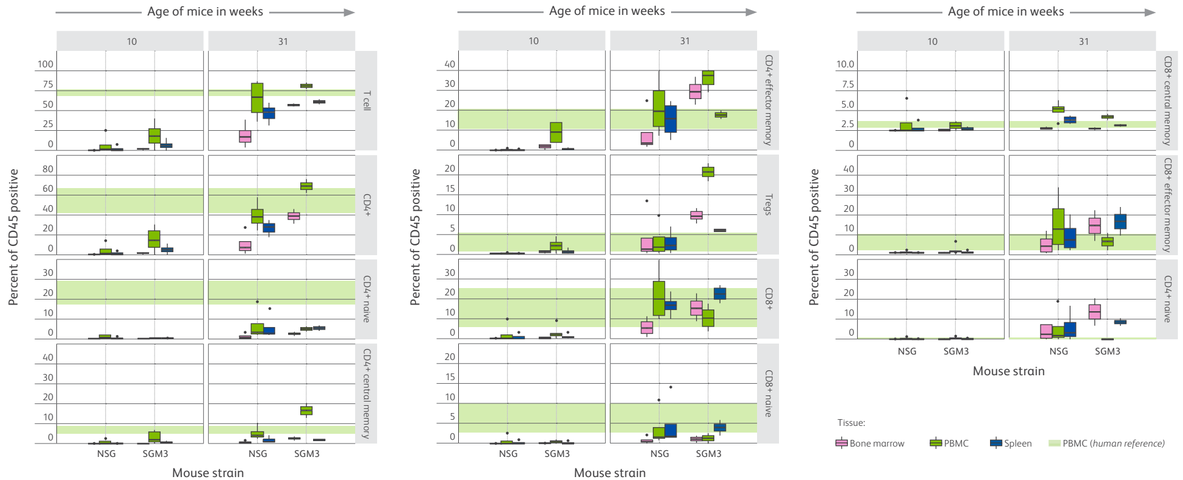


Figure 3. Sample data from T-cell panel analysis. 14-color flow cytometry panels were used on immunodeficient mice NOD scid gamma (NSG™) and triple transgenic NSG™ mice expressing human cytokines KITLG, CSF2 and IL-3 *(NSG™-SGM3)* and humanized by transplantation of human cord-blood-derived CD34+ hematopoetic stem cells. Sample tissues *(bone marrow, spleen and peripheral blood)* were stained with a 14-color antibody panel designed to enumerate T-cell subsets. The box plots show the reconstitution of various T-cell subsets in 2 mouse models at 10 and 31 weeks of age. The green bands across each plot represent the range of each subpopulation in peripheral blood as measured in normal healthy adult donors *(n = 6)* using the same panel. Mouse model and data generated in conjunction with The Jackson Laboratory.

Middlebrook AJ, et al. Comprehensive evaluation of human immune system reconstitution in NSG™ and NSG™ -SGM3 mouse models toward the development of a novel ONCO-HU™ xenograft model. BD Biosciences and The Jackson Laboratory white paper. *BD Biosciences, San Jose, CA; BD Technologies, Raleigh-Durham, NC; and The Jackson Laboratory, Bar Harbor, ME. 2017.*

BD FACSMelody™ Cell Sorter provides simple, fast and quality cell sorting ideal for enrichment of rare populations

Making the complex world of flow cytometry and sorting more accessible, enabling deep scientific insights with excellent results

Fast and efficient: Truly simple, easy to learn and maintain cell sorting. Automation enables the system to be ready in less than 17 minutes, maximizing uptime and ability to run samples sooner

Versatile: Perfect for sorting populations of cells into tubes or plates before flow cytometry analysis and scRNAseq

High performance: Excellent sensitivity for accurate results for low-density cell markers and high throughput for rare cell collection

Proven technology: Core technology derived from the highly cited BD FACSAria™ Cell Sorter

BD FACSLyric™ Flow Cytometry System is helping drive reproducibility and standardization

Trusted partner: BD, a leader in flow cytometry, continues to improve instrumentation, software and reagent solutions to help you achieve your research goals

Standardized: Easy to transfer between CROs, other manufacturing sites and collaborators with reproducible and accurate performance

Portability: Strengthen partnerships and expand global collaborations through assay portability and sharing

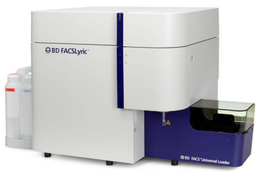
User-defined assay

Create
user-developed assay

Export
user-developed assay

Send
to collaborators

Import
user-developed assay



Better performance for better results. We've done the hard work for you.

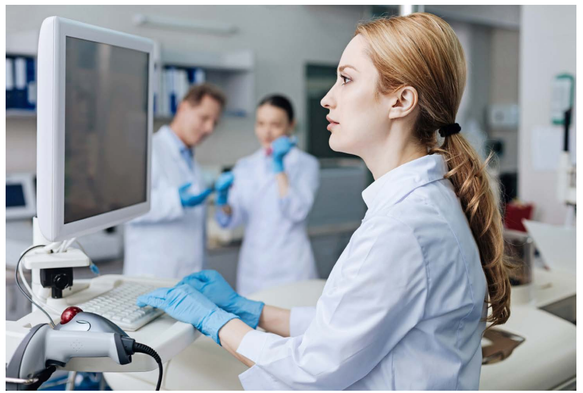
Visit **bd.com/FACSMelodySorter** and **bd.com/FACSLyric**

Attain deeper insights faster with the customizable, high parameter BD FACSymphony™ Cell Analyzer

Deep insight: Simultaneous measurement of up to 50 different characteristics of a single cell

Sensitivity: Improved detection systems enable you to identify and analyze rare cell types and events

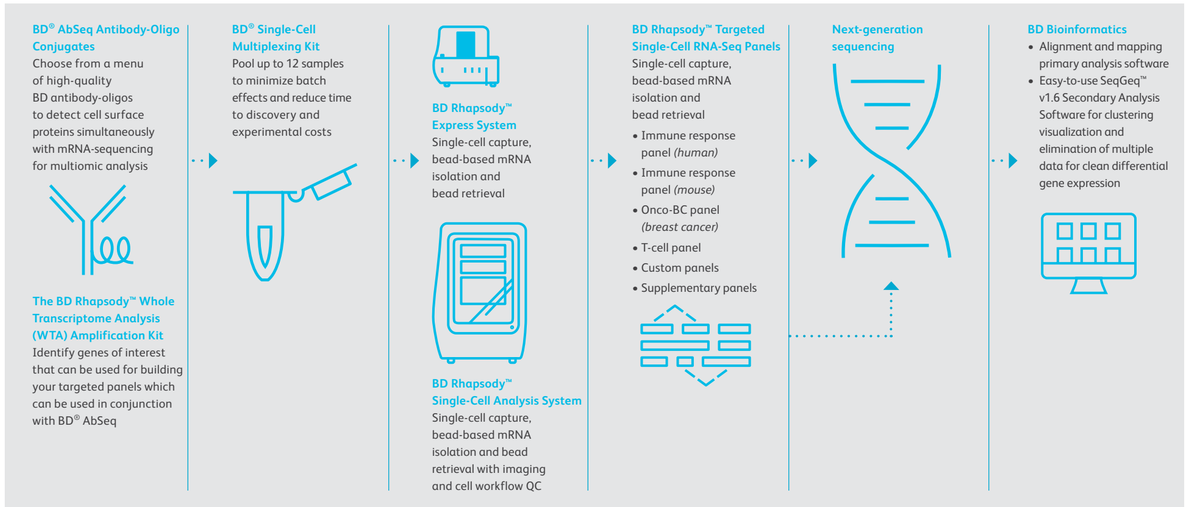
Expandable: Configured to meet the requirements of your specific research application needs today with room for growth tomorrow



Customization. High-parameter flow cytometry to fit your needs.

Visit **bd.com/FACSymphony**

Single-cell, end-to-end workflow with informatics increases the experimental power for your research



For over 40 years, BD has been a trusted partner in single-cell biology. You can rely on us to help open new frontiers in single-cell analysis.

The BD Rhapsody™ Single-Cell Analysis System with a complete set of multiomic tools, including reagents and analysis software, helps empower and streamline your research with a targeted approach

Achieve results while saving time and money with assays that dramatically reduce experimental cost and complexity, improving data and increasing efficiency of sequencing

Comprehensive: A broad multiomic solution for single-cell analysis

Synergistic: BD® AbSeq enables synergies with high-parameter flow cytometry, allowing discoveries with BD® AbSeq to transfer to panel design and WTA discoveries transfer to targeted panels with BD® AbSeq

Resolution: BD® AbSeq, when paired with WTA and mRNA targeted panels, allows for enhanced cell clustering and resolves differences between RNA and protein expression levels

"BD provides a complete range of key technologies and deep expertise to facilitate discoveries in immuno-oncology, with expanded focus on solid tumors. These include efficient single-cell dissociation from tumor tissues, the ability to generate lots of usable data from low cell numbers via high-dimensional flow cytometry or single-cell RNA sequencing, and new exciting technologies such as AbSeq."

Peter P. Lee, MD, City of Hope Comprehensive Cancer Center, Duarte, CA

Visit **bd.com/Multiomics**

The BD FACSLyric™ Flow Cytometry System identified immune checkpoint receptor expression on activated T cells

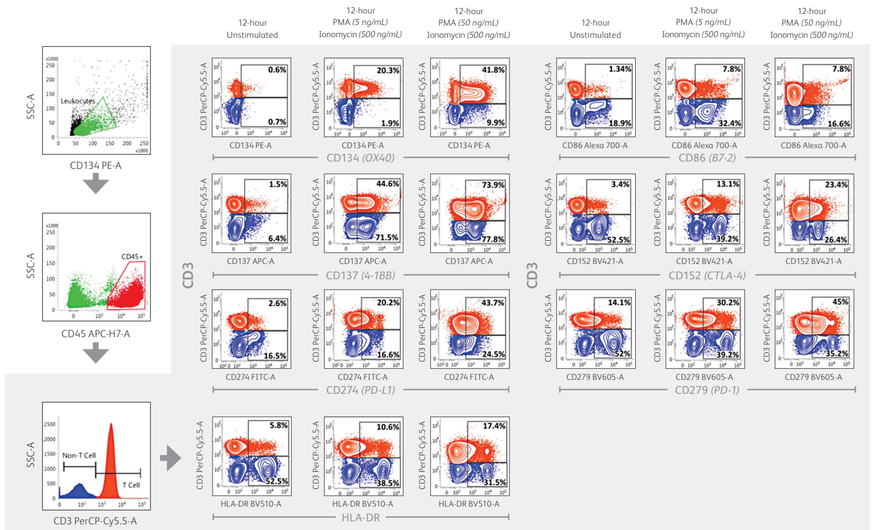


Figure 4. Demonstration of immune checkpoint receptor expression on activated T cells that are regulated in part by time-in-culture or by stimulatory conditions using BD FACSLyric™ Analyzer. Intermediate concentrations *(50 ng/mL)* of PMA plus ionomycin appeared to induce robust upregulation of CD134, CD137, PD-L1/CD274, HLA-DR, CD86, CD152 and PD-1/CD279 in CD3+ T cells.

Expression of immune checkpoint receptors on peripheral blood immune cells using a 10-color assay on the BD FACSLyric™ Flow Cytometer. Application note. *BD Biosciences, San Jose, CA. 2017.*

Target antigen	Alternate name	Clone	Fluorophore	Cat. no.
CD45	PTPRC	2D1	APC-H7	560178
CD3	n/a	SK7	PerCP-Cy™5.5	340949
HLA-DR	n/a	G46-6	BD Horizon™ BV510	563083
CD28	n/a	CD28.2	PE-Cy™7	560684
CD134	OX40	ACT35	PE	555838
CD137	4-1BB	4B4-1	APC	550890
CD274	PD-L1	M1H1	FITC	558065
CD86	B7-2	2331	Alexa Fluor® 700	561124
CD152	CTLA-4	BNI3	BD Horizon™ BV421	562743
CD279	PD-1	EH12.1	BD Horizon™ BV605	563245

Helping researchers characterize and quantify immune checkpoint receptors based on decades of deep immunology expertise.

BD FACSMelody™ Cell Sorting System provided sorting and cell isolation to enable downstream functional assessment of regulatory T cells

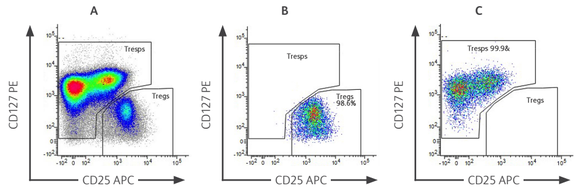


Figure 5. Sorting Treg and Tresp populations. CD4+ T cells were enriched using the BD IMag™ Human CD4 T Lymphocyte Enrichment Set-DM and stained using the Treg panel. Dead *(7-AAD+)* and other lineage-positive cells *(i.e., those expressing CD8, CD14 or CD19)* were excluded, and CD4+ T cells were identified as CD3+ CD4+ *(not shown)*. ***(A)*** Representative final sorting gates of Tregs identified as CD25 high/+ CD127 low/– and responder T cells *(Tresps)* as CD25 low/–CD127 high/+ are shown within CD4+ T cells. Sorted Tregs ***(B)*** and Tresps ***(C)*** had greater than 98% purity within the CD4+ T cell gate.

Sorting and downstream functional assessment of regulatory T cells isolating live cells with the BD FACSMelody™ Cell Sorter. BD Biosciences datasheet. *BD Biosciences, San Jose, CA. 2018.*

BD FACSMelody™ Cell Sorting System		
Cat no.	Markers	Fluorochrome
641397	CD3	APC-H7
563877	CD4	BV786
340939	CD25	APC
557938	CD127	PE
565310	CD8	PerCP-Cy™5
562692	CD14	PerCP-Cy™5
561295	CD19	PerCP-Cy™5
559925	Dead cells	7-AAD

Whether you choose one rare cell or a whole population, the BD FACSMelody™ Cell Sorter makes it easy to sort as part of your immuno-oncology workflow.

BD® AbSeq scRNAseq and protein expression levels identified T-cell subsets and rare populations of memory T cells in CAR-T infusion products

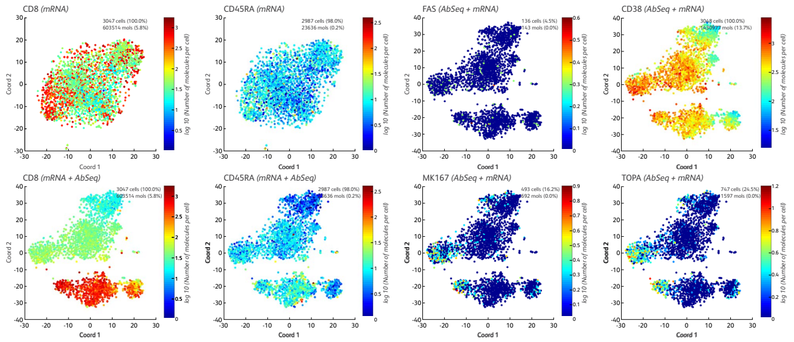


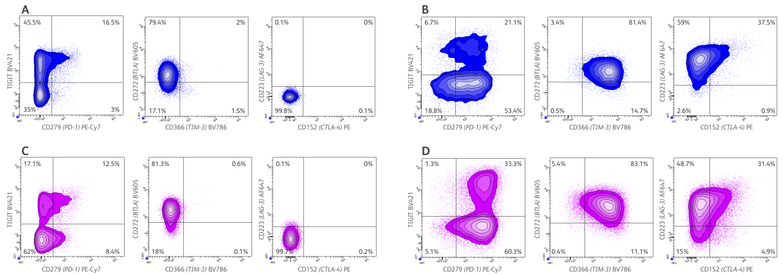
Figure 6. BD® AbSeq characterizing CAR CD19 T cells. Participants from a trial at Westmead Hospital *(Sydney, Australia)* using CAR T cells based on 4-1BB coreceptor *(instead of CD28)* and PiggyBac™ System for gene modification. Development of a single-cell multiomics approach was used to study the molecular, functional and transcriptomic profile of CAR 19 T cells in the preinfusion product and the blood of patients following adoptive transfer. This was to test the hypothesis that survival of CAR 19 T cells postinfusion is driven by long-term stem memory T cells (TSCMs).

TSCMs are identified as a minor subset within the CAR 19 T cell product and these are found expanded in the blood of patients up to 100 days post CAR 19 T cell post-infusion *(data not shown, see poster for details)*.

Cai CH, McGuire H, Clancy L, et al. Characterising the functional and genomic profiles of CAR CD19 T cells using single-cell analyses. Poster presented at ECI 2018; September 2-5, 2018; Amsterdam, The Netherlands.

BD® AbSeq resolved with higher accuracy the transcriptional and surface phenotype of CAR T cells in the infusion product than scRNAseq alone.

Multicolor flow cytometry with the BD FACSCelesta™ Flow Cytometer demonstrated comprehensive immunophenotypic analysis of exhausted T cells



Multicolor flow cytometry		
Cat. no.	Markers	Fluorochrome
560835	CD3 or live/dead cells	PerCP-Cy™5.5
559925	CD3 or live/dead cells	7-AAD
564975	CD4	APC-R700
560179	CD8	APC-H7
563963	CD45RA	BV650
561271	CD197 *(CCR7)*	FITC
746675	CD95	BV480

Multicolor flow cytometry		
Cat. no.	Markers	Fluorochrome
747844	TIGIT	BV421
555853	CD152 *(CTLA-4)*	PE
565716	CD223 *(LAG-3)*	Alexa Fluor® 647
743986	CD272 *(BTLA)*	BV605
561272	CD279 *(PD-1)*	PE-Cy™7
742857	CD366 *(TIM-3)*	BV786

Figure 7. Coexpression patterns of inhibitory receptors in unstimulated and in vitro stimulated CD8+ and CD4+ T cells. The use of bivariate plots enabled the identification of complex coexpression patterns of inhibitory receptors and highlights the heterogeneous phenotype of in vitro persistently stimulated T cells and immunophenotypic analysis of exhausted T cells. Plot analysis was performed to identify subsets of total CD8+ T and CD4+ cells coexpressing inhibitory receptors within fresh, unstimulated PBMCs and T cells persistently stimulated in vitro with Dynabeads® Human Activator CD3/CD28 and human recombinant IL-2 for 9 days. ***(A)*** Bivariate plot analysis provided information on the heterogeneity of CD8+ T cells coexpressing inhibitory receptors. For example, distinct subsets of cells expressing only TIGIT, only PD-1 or coexpressing both inhibitory receptors were detected. ***(B)*** More complex patterns of expression were observed upon persistent stimulation in vitro that resulted in differential regulation of the inhibitory receptors tested. For example, while the overall percentage of CD8+TIGIT+ cells decreased, an increase in cells coexpressing PD-1 and TIGIT was observed. Interestingly, only a small, discrete subset of CD8+ cells upregulated CTLA-4 expression, thus confirming the heterogeneity of cells persistently stimulated. ***(C–D)*** Similar observations were made for CD4+ T-cell subsets.

Evaluating the expression patterns of multiple inhibitory receptors associated with T-cell exhaustion using multicolor flow cytometry. BD Biosciences white paper. *BD Biosciences, San Jose, CA. 2018.*

Flow cytometry analysis of multiple T-cell inhibitory receptors can provide deep insight into T-cell exhaustion.

BD FACSCelesta™ Flow Cytometer with a BD® High Throughput Sampler (HTS) option helped assess PD-1 receptor occupancy

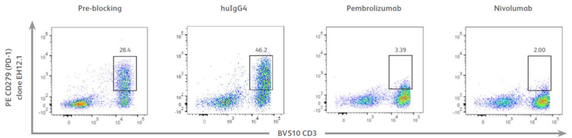


Figure 8. Assessment of PD-1 receptor occupancy in vitro after pembrolizumab or nivolumab treatment. PBMCs from one donor were stimulated overnight with 10 µg/mL of immobilized BD Pharmingen™ NA/LE Anti-Human CD3. PD-1 expression was assessed on the BD FACSCelesta™ Flow Cytometer and then nivolumab, pembrolizumab or human IgG4 isotype control was added to the cultures. The cells were cultured with the blocking anti-PD-1 antibodies as well as immobilized anti-CD3 and 10 ng/mL of BD Pharmingen™ Recombinant Human IL-2 for 3 days. The cells were washed and stained with BD Horizon™ BV510 Mouse Anti-Human CD3 and PE-EH12.1.

A high-throughput flow cytometry assay to assess PD-1 receptor occupancy. BD Biosciences datasheet. *BD Biosciences, San Jose, CA. 2018.*

Our flow cytometers can provide robust analysis of PD-1 and other checkpoint receptor expression on T-cell subsets and help with several high throughput drug-discovery applications.

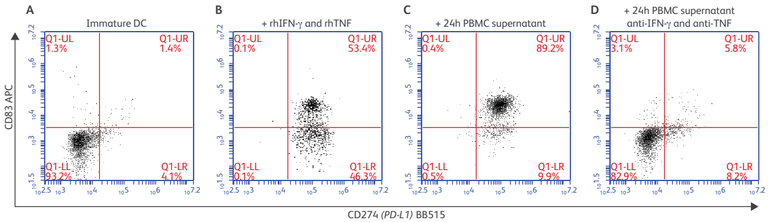
The BD Accuri™ C6 Plus Personal Flow Cytometer demonstrated stimulation of immature dendritic cells, which resulted in both cell maturation and induction of PD-L1

Figure 9. PD-L1 and CD83 expression following induction and blockage of DC maturation. Human monocyte-derived DCs were differentiated using 50 ng/mL of BD Pharmingen™ rhGM-CSF *(cat. no. 550068)* and 50 ng/mL BD Pharmingen™ rhIL-4 *(cat. no. 554605).*

(A) Immature DCs obtained on day 6 of culture did not express CD83 *(BD Pharmingen™ APC Mouse Anti-Human CD83)* or PD-L1 surface markers. ***(B–C)*** Upregulation of these markers was observed upon maturation induced by 3-day stimulation with rhIFN-γ and rhTNF or conditioned medium *(24h supernatant)* from activated PBMC cultures. ***(D)*** Cells were also stimulated with the same conditioned medium in the presence of 10 µg/mL BD Pharmingen™ Purified NA/LE Mouse Anti-Human IFN-γ *(cat. no. 554698)* and 10 µg/mL of TNF *(cat. no. 554508)* blocking antibodies, which resulted in a significant inhibition of maturation, as indicated by the lack of CD38 and PD-L1 expression.

Analysis of immuno-oncology biomarkers using personal flow cytometry. BD Biosciences datasheet. *BD Biosciences, San Jose, CA. 2018.*

This demonstrated that stimulation of immature dendritic cells ***(involved in tumor antigen presentation)*** with either conditioned medium ***(24h supernatant, plot C)*** or recombinant cytokines ***(hIFN-γ and rhTNF, plot B)*** resulted in both cell maturation ***(demonstrated by upregulation of CD83)*** and induction of PD-L1. However, adding anti-IFN-γ and anti-TNF blocking antibodies to the conditioned medium prevented upregulation of CD83 and PD-L1, and ultimately maturation of the DCs ***(plot D)***.



Several immuno-oncology biomarkers and applications can be investigated with the easy to use, simple to maintain and affordable BD Accuri™ C6 Flow Cytometer.

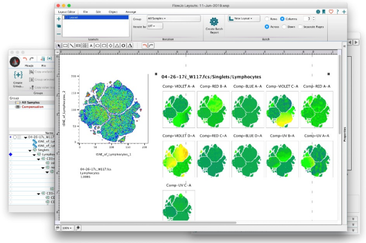
BD offers a variety of powerful software applications to help you quickly and easily analyze your data

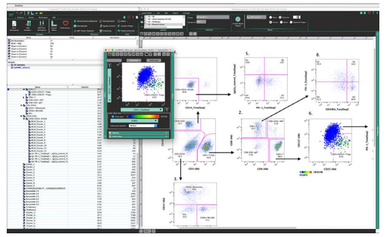
FlowJo™

Flow cytometry analysis: FlowJo™ is the leading platform for single-cell flow cytometry analysis that helps your research stand out. With the release of FlowJo™ v10.6, you can take your analysis to the next level with new features including spectral compensation, kinetic overlays, improved BD FACSDiva™ support and more.

SeqGeq™

RNAseq analysis: SeqGeq™ v1.6 is a desktop bioinformatics platform that makes complex scRNA seq analysis accessible with an intuitive interface. SeqGeq™ lets you control your analysis—no more writing R scripts to visualize your data—and easily share your results for publication and collaboration. Explore, visualize and shape your next-gen sequencing data with interactive graphs that are designed to help you focus on the insights that matter most.





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