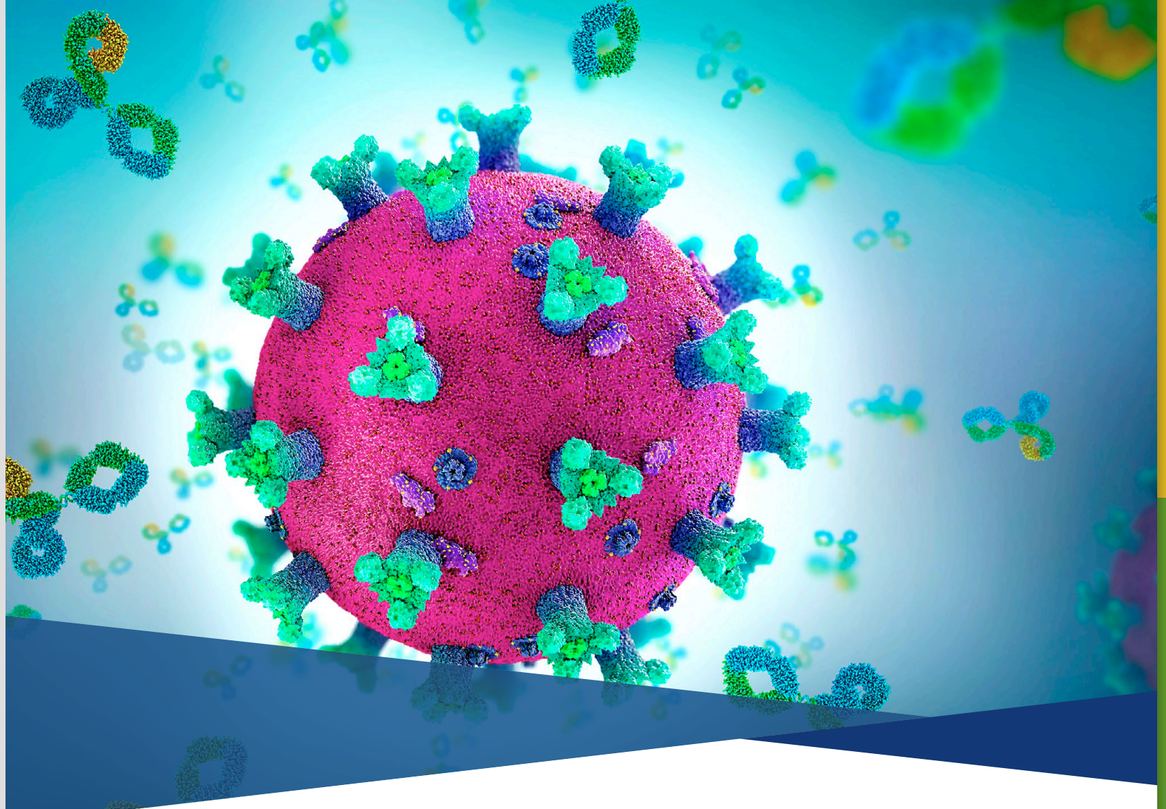


Multicolor Flow Cytometry Panels for COVID-19 Research

BD

Introduction

Protocols

Panels

As the COVID-19 pandemic continues to spread worldwide, there is a pressing need to understand the biological mechanisms determining disease progression and severity. Early research studies have reported alterations in the immune cell composition and dysfunctional immune responses in COVID-19 patients. The extent of the immune dysregulation appears to correlate with the severity of COVID-19 disease. A deeper understanding of the interactions between SARS-CoV-2 and the host immune system is required to further understand the pathogenesis of this disease and identify biomarkers that could, potentially, be used to predict, monitor and/or prevent COVID-19 progression.

Flow cytometry plays a critical role in the study of COVID-19 immune response due to the ability to simultaneously measure expression of multiple proteins at the single-cell level. Flow cytometry is therefore routinely used to assess immunophenotypic and functional changes (cytokine production, proliferation, cytotoxicity) in response to viral infection. Some of these assays are also used in vaccine research to assess vaccine candidate immunogenicity.

This document contains a collection of multicolor flow cytometry panels selected based on their relevance to the immunological changes observed in COVID-19 patients. The panels were designed using best panel design practices and their performance, defined as the ability to clearly resolve populations of interest, was tested on healthy human blood samples. For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Learn how BD provides solutions to enable your COVID-19 research

Learn about other BD technologies that further support COVID-19 research

BD® Cytometric Bead Array for multiplexed quantification of secreted cytokines

BD Rhapsody™ Single-Cell Analysis System for biomarker discovery through deep multiomic cell analysis

FlowJo™ v10 Software for simplified and advanced flow cytometry data analysis

Click on a panel of interest to view markers, reagents, experimental details and representative data:

Immunological Changes Correlated with Severe COVID-19 Manifestation	Relevant Multicolor Flow Cytometry Panels for Human Immune Cell Immunophenotyping
Reduced absolute count and/or frequency of T cells, B cells, NK cells, monocytes and/or dendritic cells[1-6,8,9,11,13]	TBMNK backbone panel
Increased frequency of memory and terminally differentiated, senescent and exhausted T cells[1,3,4,5,7,9,10,11,13]	BD Horizon™ Dri Memory T Cell Panel T cell senescence and exhaustion panel T cell inhibitory receptor panel Polyfunctional T cell panel
Altered CD4+ T cell differentiation with potential skewing toward Th17 phenotype[10]	CD4+ T cell subset panel
Increased frequency of activated T cells[1,3,10,11,13]	Activated T cell panel
Altered distribution of regulatory T cells (Tregs)[1-3,10]	Treg backbone panel CD4+ T cell subset panel
Increased frequency of plasmablasts and decreased frequency of memory B cells[11-13]	B cell subset panel
Increased frequency of exhausted and adaptive NK cells[4,7,8]	NK cell inhibitory receptor panel NK cell activating and inhibitory receptor panel
Altered distribution of monocyte subsets and decreased HLA-DR antigen density[5,6]	BD Horizon Dri Monoset Panel
Altered distribution of dendritic cell subsets (cDC1, cDC2, pDCs, CD86+ DCs)[1,12,13]	Dendritic cell subset panel

References:

1. Wang F, et al. The laboratory tests and host immunity of COVID-19 patients with different severity of illness. *JCI Insight*. 2020 May;5(10):e137799. doi: 10.1172/jci.insight.137799.
2. Chen G, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J *Clin Invest*. 2020 May;130(5):2620-2629. doi: 10.1172/JCI137244.
3. Qin C, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis*. 2020 Jul;71(15):762-768. doi: 10.1093/cid/ciaa248.
4. Zheng M, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol*. 2020 May;17(5):533-535. doi: 10.1038/s41423-020-0402-2.
5. Zhou Y, et al. Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients. *Natl Sci Rev*. 2020 June;7(6):998-1002. doi: 10.1093/nsr/nwaa041
6. Giamarellos-Bourboulis EJ, et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. *Cell Host Microbe*. 2020 June;27(6):992-1000.e3. doi: 10.1016/j.chom.2020.04.009
7. Zheng HY, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. *Cell Mol Immunol*. 2020 May;17(5):541-543. doi: 10.1038/s41423-020-0401-3
8. Maucourant C, et al. Natural killer cell immunotypes related to COVID-19 disease severity. *Sci Immunol*. 2020 August;5(50). doi: 10.1126/sciimmunol.abd6832
9. Diao B, et al. Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Front Immunol*. May 2020. doi: 10.3389/fimmu.2020.00827
10. De Biasi S, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. *Nat Commun*. 2020 July;11(1):3434. doi: 10.1038/s41467-020-17292-4
11. Mathew D, et al. Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. *Science. 2020* July;6508(369). doi: 10.1126/science.abc8511
12. Sanchez-Cerillo I, et al. COVID-19 severity associates with pulmonary redistribution of CD1c+ DC and inflammatory transitional and nonclassical monocytes. *J Clin Invest*. 2020 August;140335. doi: 10.1172/JCI140335
13. Kuri-Cervantes L, et al. Comprehensive mapping of immune perturbations associated with severe COVID-19. *Sci Immunol*. 2020 Jul;5(49):eabd7114. doi: 10.1126/sciimmunol.abd7114

The studies included here have not been independently validated by BD.
All BD reagents used in these studies are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

Learn how BD provides solutions to enable your COVID-19 research

tips & tricks

BD Pharm Lyse Lysing Buffer and BD FACS Lysing Solution work optimally when EDTA is used as an anticoagulant instead of heparin

Keep cells on ice after RBC lysis with BD FACS Lysing Solution to preserve granulocyte morphology

Perform RBC lysis with BD FACS Lysing Solution post antibody staining if antigens of interest may be impacted by a fixative

Use the BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube to facilitate the PBMC isolation workflow

tips & tricks

Consult the technical data sheet of your test size antibody to know the sample type used to predetermine the optimal concentration

If you are using a different sample type or experimental conditions, you may need to perform your own titration to determine the optimal concentration for your specific application

Watch this video to learn more about antibody titration

Protocols

Sample Type

The sample type used for a given experiment may vary depending on the cell population of interest and/or the biological application.

Sample Type	Target Populations	Protocol	Example of Applications
Whole blood	Mononuclear and polymorphonuclear cells	Red blood cell (RBC) lysis	Broad and/or high level immunophenotype of major immune cells present in blood
Peripheral blood mononuclear cells (PBMCs)	Mononuclear cells	Ficoll gradient separation	Deeper immunophenotype of major immune cells and rare subsets thereof
Enriched T cells	T cell subsets	Magnetic isolation	Immunophenotype of T cells after in vitro culture and activation

Some buffers for RBC lysis (BD FACS™ Lysing Solution) contain a fixative buffer, whereas others (BD Pharm Lyse™ Lysing Buffer) do not. The potential impact on antigen integrity and the compatibility with viability stains need to be taken into consideration when using RBC lysis solutions containing a fixative buffer.

Antibody Test Size and Titration

To save time and cell samples for researchers, pretitrated test size reagents are bottled at an optimal concentration, with the best signal to noise ratio on relevant models. Technical data sheets provide data generated on the relevant primary model at this optimal concentration.

For antibodies sold by mass (e.g., 0.2 mg/mL), antibody titration is required to determine the optimal concentration for a given cell type and application.

Learn how BD provides solutions to enable your COVID-19 research

tips&tricks

Use BD Horizon™ Brilliant Stain Buffer for optimal staining when using fluorescent dyes such as BD Horizon Brilliant™ Blue, BD Horizon Brilliant UV and/or BD Horizon Brilliant Violet™ Dyes

Use of BD Horizon Brilliant Stain Buffer Plus is recommended to reduce test volume for applications where total staining volume is a concern

Resolution of chemokine receptors may be improved by staining cells at 37° C for 10 minutes. Add the antibody cocktail for the remaining surface markers and continue with your staining protocol

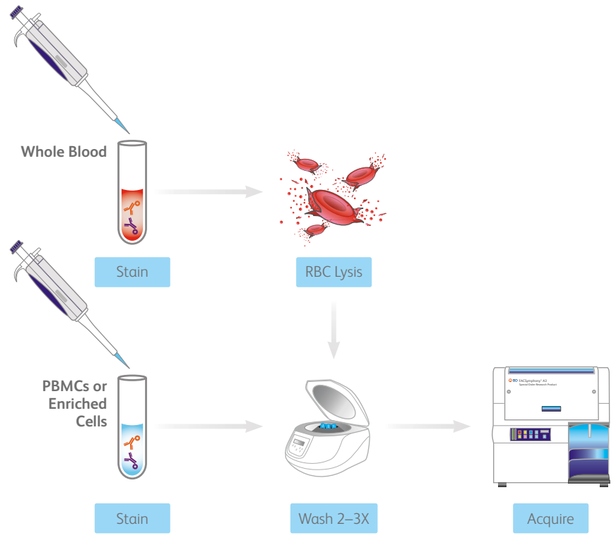
Be aware of potential adverse effects of different fixation and permeabilization buffers on surface antigens and fluorochromes

Learn more about buffer, antigen and fluorochrome compatibility

Antibody Staining

The protocols, reagents and workflow required for an optimal antibody staining depend on the cellular localization of the antigen of interest, i.e., surface or intracellular markers, as well as the impact of buffers on antigen and fluorochrome integrity.

Workflow for human surface marker staining:



If analysis of both surface and intracellular (IC) markers is required, at least two workflows can be used depending on the antigens of interest and the selected fluorochromes.

Workflow	Protocol	Recommended When	Watch-Out
Sequential stain	Surface stain » Fix/perm » IC stain	Antigen integrity and antibody binding may be impacted by the selected fix/perm buffers	Fluorochrome performance may be impacted by the selected fix/perm buffers
Simultaneous stain	Fix/perm » Surface + IC stain	Fluorochrome performance may be impacted by the selected fix/perm buffer	Antigen integrity and antibody binding may be impacted by the selected fix/perm buffers

tips&tricks

Be aware of potential downregulation/ internalization of some surface markers (i.e., CD3, CD4) when using stimuli such as phorbol 12-myristate 13-acetate (PMA)

Titer the concentration of stimulus and incubation time to ensure resolution of potentially impacted surface markers and sufficient cytokine production

Use bright fluorochrome and/or perform intracellular staining for downregulated and/or internalized markers

Explore different activation protocols to meet your needs

tips&tricks

Let cells incubate or culture with the stimulus for at least an hour before adding the protein transport inhibitor

Be aware of cell toxicity upon prolonged exposure to protein transport inhibitors (>18 hours)

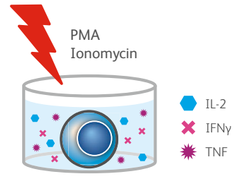
If prolonged incubation time is required, use the less toxic BD GolgiPlug Inhibitor

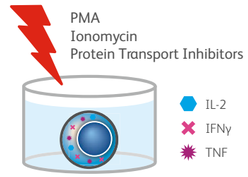
When investigating multiple cytokines, cells can be incubated with both BD GolgiStop and BD GolgiPlug Inhibitors for optimal resolution of each cytokine

Learn more about intracellular flow cytometry

Cell Activation

Immune cell activation is often required to induce cytokine production and detection via flow cytometry. The protocols and reagents for cell activation vary depending on the cytokine and species of interest.





Intracellular Cytokine Staining

Protein transport inhibitors may be required to trap cytokines inside the cells prior to intracellular cytokine analysis. The choice between two common protein transport inhibitors, monensin (BD GolgiStop™) and brefeldin A (BD GolgiPlug™), as well as the incubation time, depend on the cytokine and species of interest.

Species	Cytokines	Protein Transport Inhibitor
Human	IL-1α, IL-6, IL-8, TNF	BD GolgiStop
Human	IFN-γ, IL-2, IL-10, IL-12, MCP-1, MCP-3, MIG, MIP-1α, RANTES	Either BD GolgiStop or BD GolgiPlug
Mouse	IL-6, IL-12, TNF	BD GolgiPlug
Mouse	GM-CSF, IL-3, IL-4, IL-5, IL-10	BD GolgiStop
Mouse	IFN-γ, IL-2	Either BD GolgiStop or BD GolgiPlug

tips&tricks

Perform stain with FVS before fixation in protein-free buffer (e.g., 1X PBS) to avoid dye sequestration and suboptimal staining

Wash FVS stained cells with protein containing buffer (e.g., 1X PBS with FBS or BSA) to eliminate unbound dye and reduce background

FVS titration is recommended for specific cell types and/or applications

Learn more about nucleic acid stains

Learn more about FVS

tips&tricks

When using BD Trucount Absolute Counting Tubes, use a buffer with protein in it (e.g., 1X PBS with FBS or BSA) to avoid cell clumping and inaccurate counts

Stain whole blood by following a lyse/no-wash procedure when using BD Trucount Absolute Counting Tubes to avoid potential cell loss and inaccurate counts

Antigen quantification using the BD Quantibrite PE Phycoerythrin Fluorescence Quantitation Kit is based on the assumption of a fluorochrome-to-antibody ratio of 1:1

Learn how BD provides solutions to enable your COVID-19 research

Viability Stains

Viability stains enable exclusion of dead cells that could introduce staining artifacts or alter protein expression patterns. This is particularly important when analyzing samples with high amounts of dead cells, such as activated or tissue-derived cells. The choice between two main categories of viability stains, nucleic acid stains and fixable viability stains (FVS), depends on the experimental workflow.

Viability Stain Family	Viability Stain Examples	Assay Type	Experimental Workflow
Nucleic acid stains	BD Pharmingen™ 7-AAD BD Pharmingen DAPI Solution BD Via-Probe™ Green Nucleic Acid Stain BD Via-Probe Red Nucleic Acid Stain	No wash	Surface marker staining
Fixable viability stains (FVS)	BD Horizon Fixable Viability Stain 440UV BD Horizon Fixable Viability Stain 450 BD Horizon Fixable Viability Stain 510 BD Horizon Fixable Viability Stain 520 BD Horizon Fixable Viability Stain 570 BD Horizon Fixable Viability Stain 575V BD Horizon Fixable Viability Stain 620 BD Horizon Fixable Viability Stain 660 BD Horizon Fixable Viability Stain 700 BD Horizon Fixable Viability Stain 780	Wash	Intracellular and/or surface marker staining

Absolute Cell Count and Antigen Quantification

Flow cytometry analysis conventionally provides information about the relative frequency of populations of interest and intensity of fluorescent signal (median fluorescence intensity, MFI). The BD Trucount™ Absolute Counting Tubes and BD Quantibrite™ PE Phycoerythrin Fluorescence Quantitation Kit are companion products developed specifically to enable absolute cell count and surface antigen quantification, respectively.

Detailed Protocols and Technical Data Sheets (TDS)

Immunofluorescence surface marker staining of whole blood using a lyse/wash procedure	View protocol
Direct immunofluorescence staining of whole blood using a lyse/no-wash procedure	View protocol
Immunofluorescence surface marker staining of PBMCs	View protocol
Immunofluorescence staining of intracellular cytokines	View protocol
Biosafety and sample handling guide	View protocol
BD Horizon Brilliant Stain Buffer	View TDS
BD Pharmingen Leukocyte Activation Cocktail	View TDS
BD GolgiPlug Protein Transport Inhibitor	View TDS
BD GolgiStop Protein Transport Inhibitor	View TDS
BD Via-Probe Green Nucleic Acid Stain	View TDS
BD Horizon Fixable Viability Stain 575V	View TDS
BD Trucount Absolute Counting Tubes	View TDS
BD Quantibrite PE Phycoerythrin Fluorescence Quantitation Kit	View TDS

Learn how BD provides solutions to enable your COVID-19 research

Explore the possibility to manufacture your multicolor panel of choice into a dried BD Horizon Dri Chroma Cocktail for ease of use and standardization

Learn more about the advantages of BD Horizon Dri Small Batch Panels

Panels

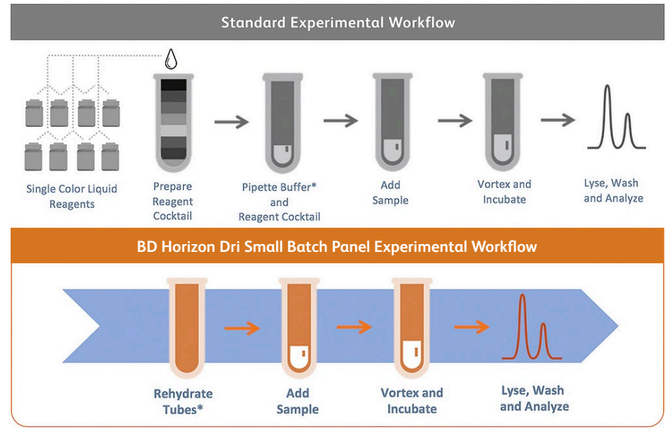
Predesigned Panels to Accelerate Your Research

The multicolor panels described in this document have been designed to immunophenotype major immune cell subsets involved in viral response and reported to be altered in severe manifestations of COVID-19. These panels have been created following the best practices for panel design. Panel performance, defined as the ability to clearly resolve populations of interest, was tested on peripheral blood from healthy donors.

Different types of panels are available to meet different needs, including panel design flexibility, improved workflow and standardization capabilities.

Multicolor Panels	Backbone Panels	BD Horizon™ Dri Small Batch Panels
Liquid	Liquid	Dried
Individual vials for each marker	Individual vials for each marker	Preformulated cocktail tubes
Optimal combination of fluorochromes and markers defining immune cells of interest	Open drop-in channels specifically selected to allow panel expansion with minimal spread introduction	Predefined, unit-sized dried cocktails
Save time and effort for marker selection and panel design	Minimize the risk of losing resolution when expanding your panel	Accelerate workflow and improve standardization

Workflow improvement using BD Horizon Dri Small Batch Panels



*If cocktail contains one or more BD Horizon Brilliant Dye, rehydrate tubes using the BD Horizon Brilliant Stain Buffer (BSB)

Learn how BD provides solutions to enable your COVID-19 research

Contact us to purchase or modify this panel to meet your experimental needs

Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

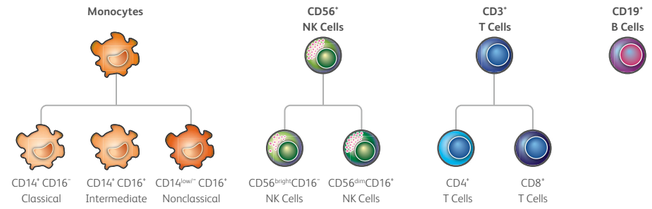
Panel performance may vary when using instruments different from the one used in this experiment

These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

TBMNK Backbone Panel

The TBMNK backbone panel is a 7-color flow cytometry panel designed to identify human total $CD3^+$ T cells, $CD4^+$ and $CD8^+$ T cell subsets, total $CD19^+$ B cells, $CD56^{bright}CD16^-$ and $CD56^{dim}CD16^+$ NK cell subsets, $CD14^+CD16^-$ classical, $CD14^+CD16^+$ intermediate and $CD14^{low/-}CD16^+$ nonclassical monocyte subsets.

Selected channels were left open for drop-in addition to facilitate deeper characterization of a lineage of interest or virus-specific immune cells. The drop-in channels were strategically selected based on fluorochrome spectral properties to minimize resolution impact between the backbone panel and the drop-ins.



Backbone Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	CD3	BV510	UCHT1	5 µL	563109
	CD56	BV711	NCAM16.2	5 uL	563169
	CD14	BV786	M5E2	5 uL	563698
Blue 488 nm	CD4	BB700	SK3	5 µL	566392
	CD16	PE-Cy™7	B73.1	5 µL	335788
Red 640 nm	CD19	APC-R700	HIB19	5 µL	564977
	CD8	APC-H7	SK1	5 µL	641400

Drop-Ins—T Cell Subsets*

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	CD95	BV421	DX2	5 µL	562616
Blue 488 nm	CD57	FITC	NK-1	2.5 µL	555619
	CD197 (CCR7)	PE	2-L1-A	5 µL	566741
Red 640 nm	CD45RA	APC	HI100	20 µL	550855

Drop-Ins—B Cell Subsets*

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	CD38	BV421	HIT2	5 µL	562444
Blue 488 nm	IgD	BB515	IA6-2	5 µL	565243
	CD24	PE	ML5	20 µL	555428
Red 640 nm	CD27	APC	M-T271	20 µL	558664

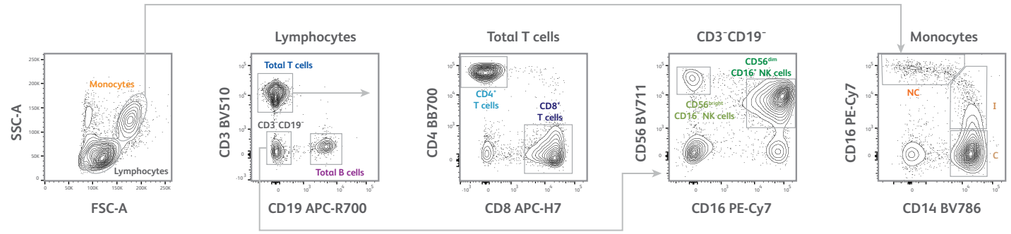
*Examples of drop-ins compatible with the backbone panel. These drop-in combinations have not been tested.

Experimental Information

Sample Type	PBMCs
Panel Type	Backbone panel
Protocols	PBMC isolation Surface marker staining
Panel Tested On	3-laser, 12-color (5 violet/4 blue/3 red) BD FACSLyric™ Flow Cytometer

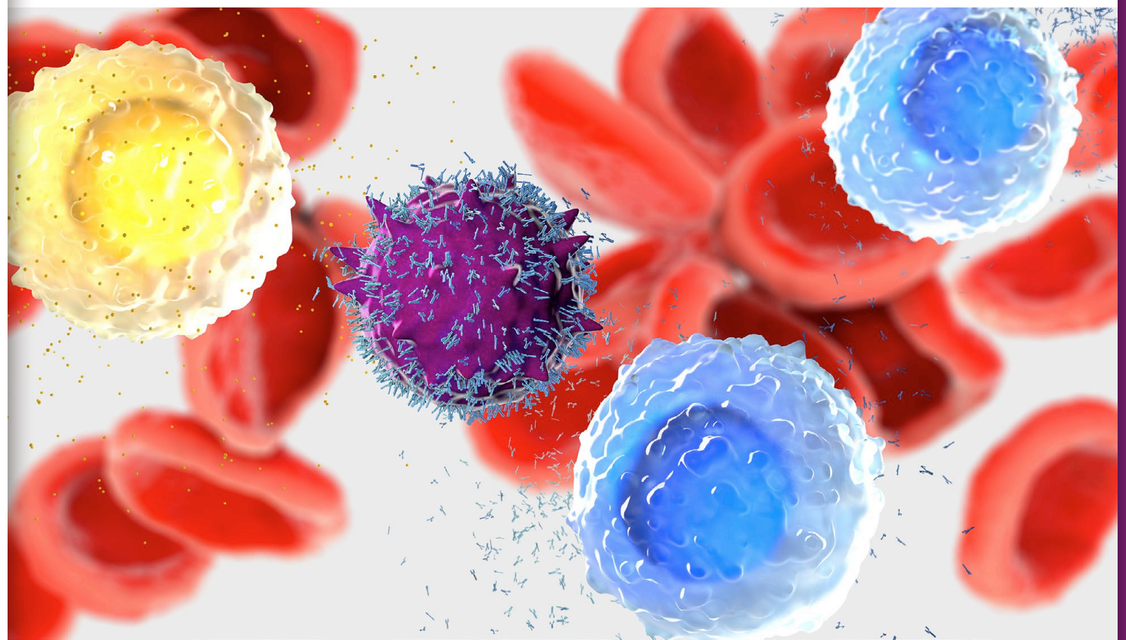
Companion Products

Product	Application	Catalog Number
BD Horizon Brilliant Stain Buffer	Optimal stain conditions	563794



Identification of major human immune subsets using the TBMNK backbone panel

Representative analysis of PBMCs from healthy human subjects (N = 5). Lymphocytes and monocytes were identified based on light scatter properties. From the lymphocytes gate, total T and B cells were detected as $CD3^+$ and $CD19^+$ cells, respectively. From the total T cells gate, $CD4^+$ and $CD8^+$ T cell subsets could be further identified. From the $CD3^-CD19^-$ gate, two NK cell subsets were detected as $CD56^{bright}CD16^-$ and $CD56^{dim}CD16^+$ cells. From the monocytes gate, three subsets were defined as $CD14^+CD16^-$ classical (C), $CD14^+CD16^+$ intermediate (I) and $CD14^{low/-}CD16^+$ nonclassical (NC) monocytes. Samples were acquired on a 3-laser, 12-color BD FACSLyric Flow Cytometer. Data analysis was performed using FlowJo™ v10 Software. The FITC, PE, BV421 and APC channels were left open for the addition of drop-ins of interest with minimal resolution impact between the backbone panel and the drop-ins.



Learn more about BD Horizon Dri Small Batch Panels

You may be also interested in the T cell senescence and exhaustion panel

Contact us to purchase or modify this panel to meet your experimental needs

Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

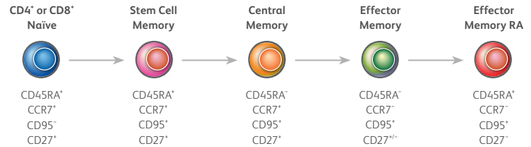
Panel performance may vary when using instruments different from the one used in this experiment

These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

BD Horizon Dri Memory T Cell Panel

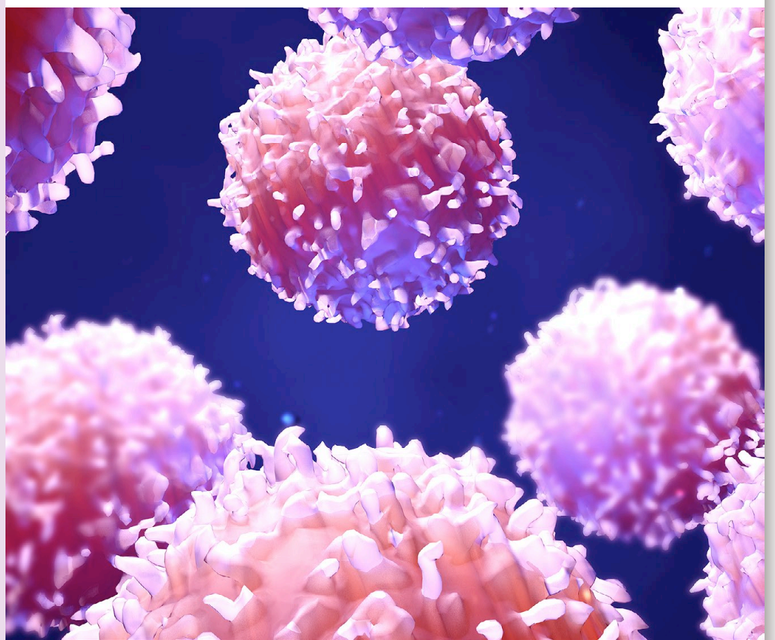
The BD Horizon Dri Memory T Cell Panel is a 7-color flow cytometry panel designed to identify major subsets of human $CD4^+$ or $CD8^+$ T cells, including naïve, stem cell memory, central memory, effector memory and terminally differentiated effector memory re-expressing CD45RA (EMRA) T cells.

This panel is available as unit sized, preformulated and performance-optimized dried cocktail.



Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Tests Per Kit
Violet 405 nm	CD4	V450	SK3	5
	CD8	V500-C	SK1	
	CD45RA	BV605	HI100	
Blue 488 nm	CD197 (CCR7)	FITC	150503	
	CD95	PE-Cy7	DX2	
Red 640 nm	CD27	APC-R700	M-T271	
	CD3	APC-H7	SK7	

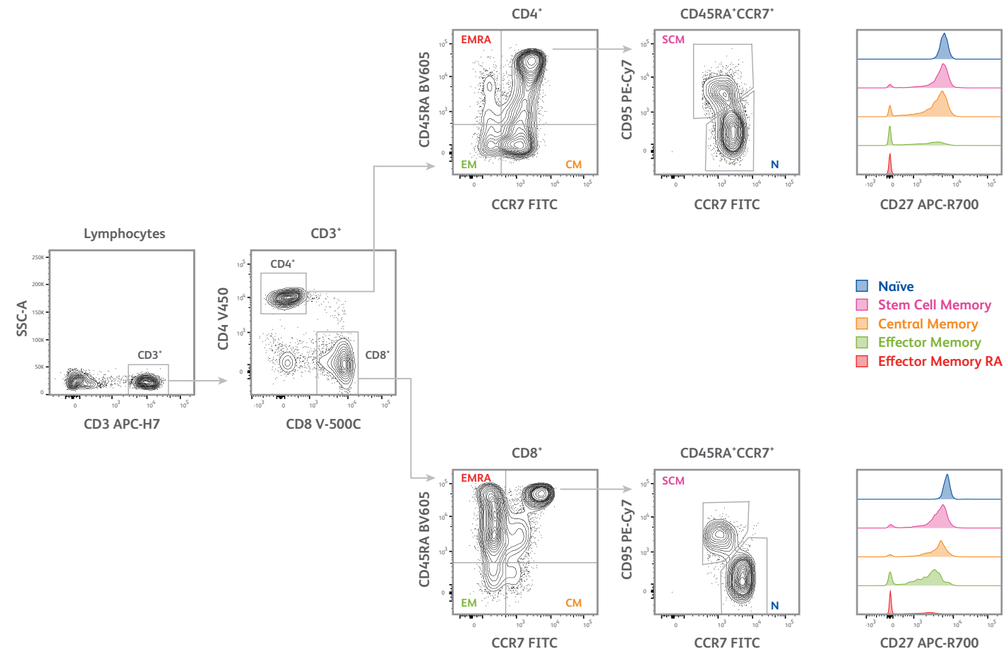


Experimental Information

Sample Type	Whole blood
Panel Type	BD Horizon Dri Small Batch Panel
Protocols	Surface marker staining Red blood cell lysis (lyse/wash)
Panel Tested On	3-laser, 12-color (5 violet/4 blue/3 red) BD FACSLyric Flow Cytometer

Companion Products

Product	Application	Catalog Number
BD Horizon Brilliant Stain Buffer	Optimal stain conditions	563794
BD FACS™ Lysing Solution	Red blood cell lysis	349202



Identification of naïve and memory T cell subsets using the BD Horizon Dri Memory T Cell Panel

Representative analysis of whole blood from healthy human subjects (N = 3). After surface marker staining, cells were lysed with BD FACS Lysing Solution. Lymphocytes were first identified based on light scatter properties (not shown). From the lymphocyte gate, $CD3^+$ total T cells and $CD4^+$ and $CD8^+$ subsets thereof were then defined. From either $CD4^+$ or $CD8^+$ T cell gate, central memory (CM), effector memory (EM) and effector memory RA (EMRA) subsets were identified based on differential expression of CD45RA and CD197 (CCR7). From the $CD45RA^+CCR7^+$ gate, $CD95^+$ stem cell memory and $CD95^-$ naïve T cells were further identified. The histogram overlays show the different patterns of CD27 expression within the distinct T cell subsets. The BD Horizon Dri Memory T Cell Panel showed equivalent performance relative to its counterpart liquid panel (not shown). Samples were acquired on a 3-laser, 12-color BD FACSLyric Flow Cytometer. Data analysis was performed using FlowJo v10 Software.

You may also be interested in the BD Horizon Dri Memory T Cell Panel

Contact us to purchase or modify this panel to meet your experimental needs

Learn how BD provides solutions to enable your COVID-19 research

Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

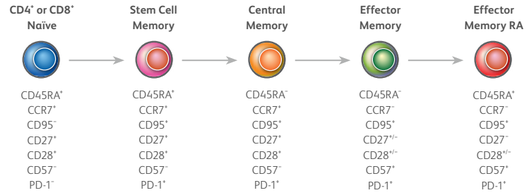
Panel performance may vary when using instruments different from the one used in this experiment

These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

T Cell Senescence and Exhaustion Panel

The T cell senescence and exhaustion panel is a 10-color flow cytometry panel designed to identify major subsets of human CD4+ or CD8+ T Cells, including naïve, stem cell memory, central memory, effector memory, terminally differentiated effector memory T cell re-expressing CD45RA (EMRA), CD57+ senescent and PD-1+ exhausted T cells.

Two panels with same specificities, but different fluorochrome combinations, were designed for compatibility with either 3-laser (5 violet, 4 blue, 3 red) or 5-laser (4 UV, 6 violet, 2 blue, 3 yellow-green, 3 red) instrument configurations.



3-Laser Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	CD279 (PD-1)	BV421	EH12.1	5 µL	562516
	CD28	BV480	CD28.2	5 µL	566110
	CD27	BV605	M-T271	1.25 µL	740398
	CD8	BV786	RPA-T8	5 µL	563823
Blue 488 nm	CD57	FITC	NK-1	2.5 µL	555619
	CD95	PE	DX2	20 µL	555674
	CD45RA	PE-Cy7	HI100	5 µL	560675
Red 640 nm	CD197 (CCR7)	APC	2-L1-A	5 µL	566762
	CD4	Alexa Fluor® 700	SK3	5 µL	566318
	CD3	APC-H7	SK7	5 µL	560176

5-Laser Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Ultraviolet 355 nm	CD3	BUV395	SK7	5 µL	564001
	CD4	BUV496	SK3	5 µL	612936
	CD27	BUV615	M-T271	1.25 µL	751135
Violet 405 nm	CD279 (PD-1)	BV421	EH12.1	5 µL	562516
	CD45RA	BV480	HI100	5 µL	566114
	CD8	BV750	RPA-T8	1.25 µL	747385
Blue 488 nm	CD57	FITC	NK-1	2.5 µL	555619
Yellow-Green 561 nm	CD95	PE	DX2	20 µL	555674
	CD28	PE-Cy7	CD28.2	5 µL	560684
Red 640 nm	CD197 (CCR7)	APC	2-L1-A	5 µL	566762

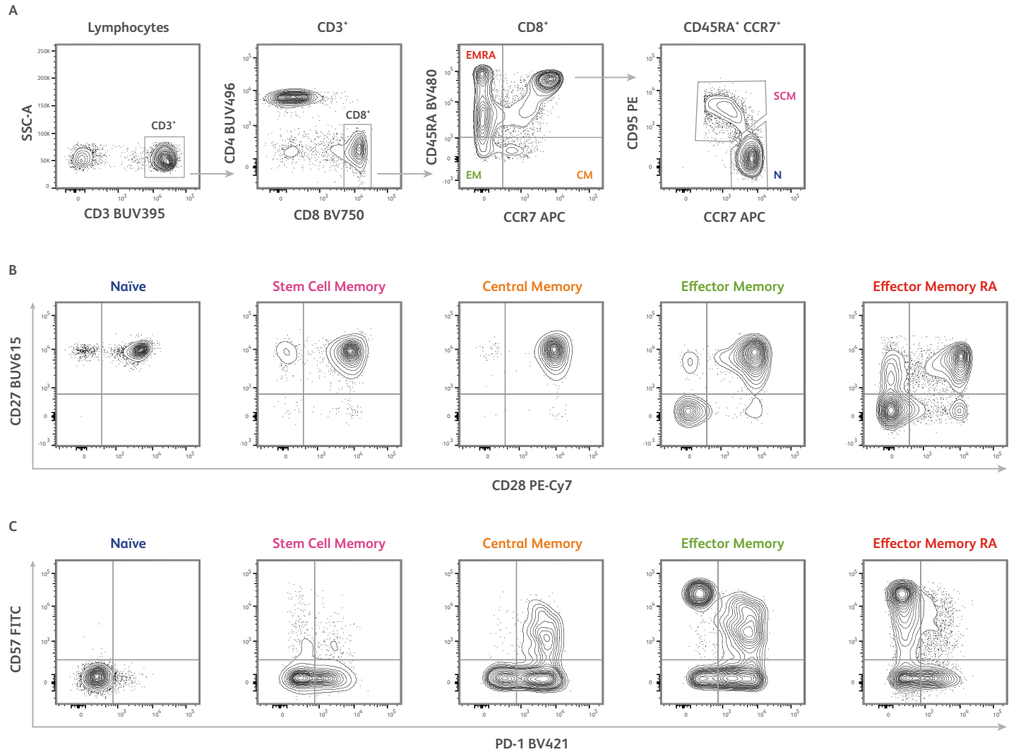
Experimental Information

Sample Type	PBMCs
Panel Type	Multicolor panel
Protocols	PBMC isolation Surface marker staining
3-Laser Panel Tested On	3-laser, 12-color (5 violet/4 blue/3 red) BD FACSCelesta™ Flow Cytometer*
5-Laser Panel Tested On	5-laser, 18-color (4 UV/6 violet/2 blue/3 yellow-green/3 red) BD LSRFortessa™ X-20 Flow Cytometer

*Filter configuration was modified to use PE-Cy7 in place of PE-CF®594

Companion Products

Product	Application	Catalog Number
BD Horizon Brilliant Stain Buffer	Optimal stain conditions	563794



Identification of differentiated, senescent and exhausted human T cell subsets using the T cell senescence and exhaustion panel

Representative analysis of CD8+ T cells from PBMCs isolated from healthy human subjects (N = 3). Lymphocytes were first identified based on light scatter properties (not shown). **A**) From the lymphocyte gate, CD3+ total T cells and CD8+ subset thereof were then defined. From the CD8+ T cells gate, central memory (CM), effector memory (EM) and effector memory RA (EMRA) subsets were identified based on differential expression of CD45RA and CD197 (CCR7). From the CD45RA+CCR7+ gate, CD95+ stem cell memory and CD95- naïve T cells were further identified. **B-C**) The expression of CD27, CD28, CD57 and PD-1 was assessed within each indicated T cell subset. As expected, T cell differentiation was marked by a progressive downregulation of CD27 and CD28 (**B**), and upregulation of CD57 and PD-1 (**C**). Samples were acquired on a 5-laser, 18-color BD LSRFortessa X-20 Flow Cytometer. Data analysis was performed using FlowJo v10 Software. A panel enabling resolution of the same T cell subsets was also designed for and tested on a 3-laser, 12-color BD FACSCelesta Flow Cytometer.

You may also be interested in the polyfunctional T cell panel

Contact us to purchase or modify this panel to meet your experimental needs

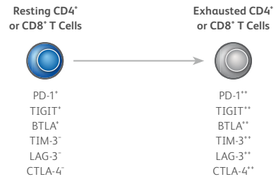
Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

Panel performance may vary when using instruments different from the one used in this experiment

These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

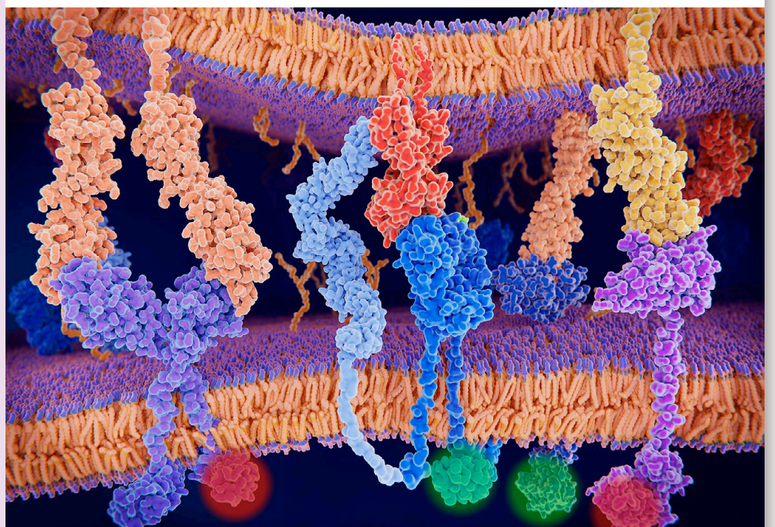
T Cell Inhibitory Receptor Panel

The T cell inhibitory receptor panel is a 12-color flow cytometry panel designed to simultaneously measure the expression of six inhibitory receptors (PD-1, CTLA-4, TIM-3, LAG-3, BTLA, TIGIT) in either human CD4⁺ or CD8⁺ T cells. This panel enables a detailed assessment of multiple markers associated with T cell exhaustion. The presence of antibodies for the detection of CD45RA, CD197 (CCR7) and CD95 further allows the assessment of these inhibitory receptor expression patterns within subsets of naïve, stem cell memory, central memory, effector memory and terminally differentiated effector memory T cell re-expressing CD45RA (EMRA).



Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	TIGIT	BV421	741182	2.5 µL	747844
	CD95	BV480	DX2	2.5 µL	746675
	CD45RA	BV605	HI100	5 µL	562886
	CD272 (BTLA)	BV711	J168-540	5 µL	743987
	CD366 (TIM-3)	BV786	TD3	2.5 µL	742857
Blue 488 nm	CD197 (CCR7)	FITC	150503	5 µL	561271
	CD152 (CTLA-4)	PE	BNI3	20 µL	555853
	CD3	PerCP-Cy5.5	UCHT1	5 µL	560835
	CD279 (PD-1)	PE-Cy7	EH12.1	5 µL	561272
Red 640 nm	CD223 (LAG-3)	Alexa Fluor 647	T47-530	5 µL	565716
	CD8	Alexa Fluor 700	RPA-T8	5 µL	561453
	CD4	APC-H7	RPA-T4	5 µL	560158



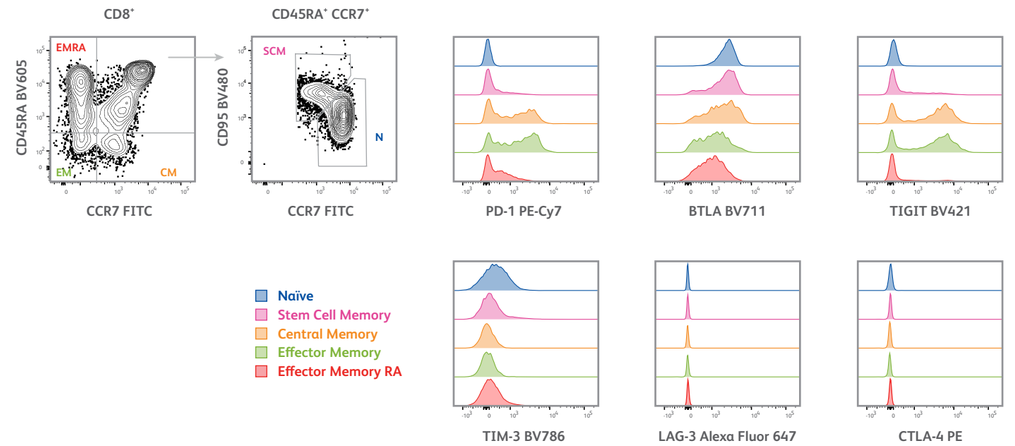
Experimental Information

Sample Type	Enriched and activated T cells
Panel Type	Multicolor panel
Protocols	PBMC isolation T cell magnetic enrichment T cell activation Surface marker staining
Panel Tested On	3-laser, 12-color (5 violet/4 blue/3 red) BD FACSLyric Flow Cytometer

Companion Products

Product	Application	Catalog Number
BD IMag™ Human T Lymphocyte Enrichment Set-DM	T cell isolation	557874
BD Horizon Brilliant Stain Buffer	Optimal stain conditions	563794

A



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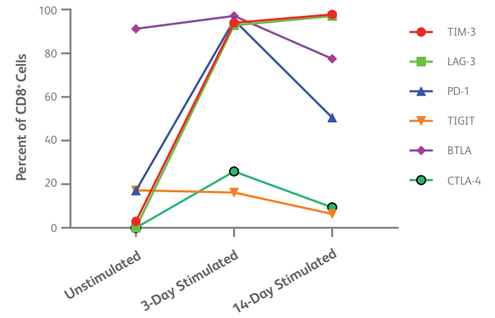


Analysis of the expression of markers associated with T cell exhaustion using the T cell inhibitory receptor panel

Representative analysis of human T cells unstimulated or stimulated with Dynabeads™ Human T-Activator CD3/CD28 (Thermo Fisher Scientific). T cells were isolated from healthy donors (N = 2) using BD IMag T Lymphocyte Magnetic Enrichment Set-DM. **A**) Distribution of inhibitory receptor expression in resting CD8⁺ T cells. After gating on CD8⁺CD4⁻ T cells, distinct subsets of naïve (N), stem cell memory (SCM), effector memory (EM) and effector memory RA (EMRA) were defined based on CD45RA, CCR7 and CD95 expression. Expression patterns of the inhibitory receptors PD-1, BTLA, TIGIT, TIM-3, LAG-3 and CTLA-4 were assessed in each T cell subset. **B**) Kinetic expression of inhibitory receptors in total CD8⁺ T cells over 14 days of chronic stimulation. Samples were acquired on a 3-laser, 12-color BD FACSLyric Flow Cytometer. Data analysis was performed using FlowJo v10 Software. The graph was created using GraphPad Prism 8.

You may also be interested in the T cell inhibitory receptor panel

Contact us to purchase or modify this panel to meet your experimental needs

Learn how BD provides solutions to enable your COVID-19 research

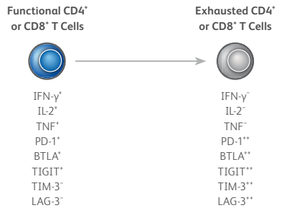
Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

Panel performance may vary when using instruments different from the one used in this experiment

These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

Polyfunctional T Cell Panel

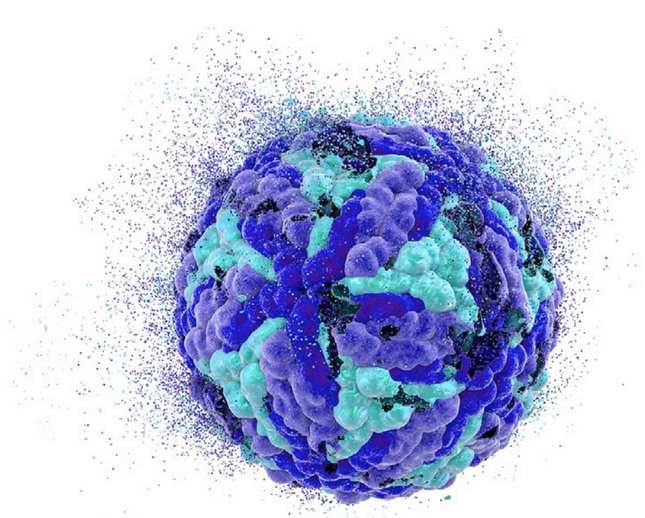
The polyfunctional T cell panel is an 11-color flow cytometry panel designed for the simultaneous assessment of the production of three major inflammatory cytokines (IFN-γ, TNF and IL-2) by either human CD4+ or CD8+ T cells. The panel further allows the measurement of five inhibitory receptors (PD-1, BTLA, TIGIT, TIM-3, LAG-3). This panel enables the combinatorial analysis of cytokine production and inhibitory receptor expression, which correlate with T cell function.



Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	TIGIT	BV421	741182	2.5 µL	747844
	CD223 (LAG-3)	BV480	T47-530	5 µL	746609
	CD272 (BTLA)	BV711	J168-540	5 µL	743987
	CD366 (TIM-3)	BV786	TD3	2.5 µL	742857
Blue 488 nm	IFN-γ	FITC	B27	0.6 µL	554700
	IL-2	PE	MQ1-17H12	20 µL	559334
	Viability	FVS620*	N/A	1 µL	564996
	CD279 (PD-1)	PE-Cy7	EH12.1	5 µL	561272
Red 640 nm	TNF	APC	MAb11	20 µL	551384
	CD4	APC-R700	RPA-T4	5 µL	564975
	CD8	APC-H7	SK1	5 µL	560179

* When excited off the blue laser, the fixable viability stain FVS620 is detected in the same channel used for detection of PerCP-Cy5.5



Experimental Information

Sample Type	Enriched and activated T cells
Panel Type	Multicolor panel
Protocols	PBMC isolation T cell magnetic enrichment T cell activation Surface and intracellular marker staining Fixable viability staining
Panel Tested On	3-laser, 12-color (5 violet/4 blue/3 red) BD FACSLyric Flow Cytometer

Companion Products

Product	Application	Catalog Number
BD IMag Human T Lymphocyte Enrichment Set-DM	T cell isolation	557874
BD Horizon Brilliant Stain Buffer	Optimal stain conditions	563794
BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit	Fixation and permeabilization for intracellular staining	554714
BD GolgiPlug™ Protein Transport Inhibitor (brefeldin A)	Intracellular cytokine detection	555029
BD GolgiStop™ Protein Transport Inhibitor (monensin)	Intracellular cytokine detection	554724

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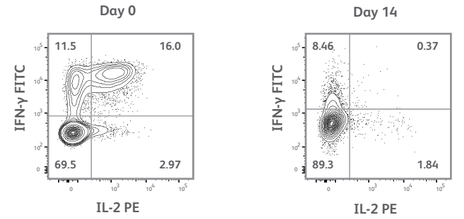

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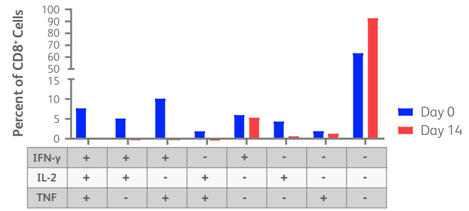

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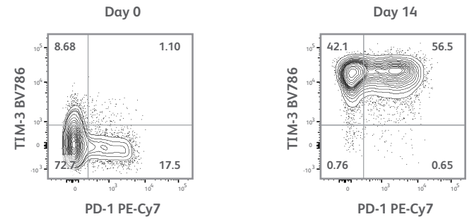

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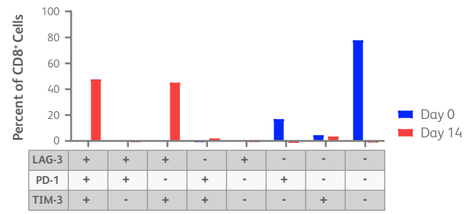


Simultaneous assessment of cytokine production and inhibitory receptor expression using the polyfunctional T cell panel

Representative analysis of human freshly isolated T cells (day 0) and T cells activated with Dynabeads Human T-Activator CD3/CD28 (day 14) after 5-hour stimulation with PMA/Ionomycin. Cells were isolated from healthy human subjects (N = 2) using BD IMag T Lymphocyte Magnetic Enrichment Set-DM. Data show analysis performed on gated $CD8^+CD4^-$ T cells. The panel allows similar analysis of $CD4^+CD8^-$ cells. **A-B**) Chronic 14-day stimulation resulted in a dramatic reduction in $CD8^+$ T cells producing inflammatory cytokines IFN-γ and IL-2 (**A**) and upregulation of inhibitory receptors PD-1 and TIM-3 (**B**). **C-D**) Boolean gates were created to perform combinatorial expression analysis of three inflammatory cytokine (IFN-γ, TNF and IL-2) and three inhibitory receptors (PD-1, LAG-3 and TIM-3). Data show decrease in $CD8^+$ T cells simultaneously producing 2–3 cytokines (**C**) and increase in cells simultaneously expressing 2–3 inhibitory receptors (**D**) in cells chronically stimulated for 14 days (red bars), as compared to freshly isolated T cells (day 0, blue bars). Samples were acquired on a 3-laser, 12-color FACSLyric Flow Cytometer. Data analysis was performed using FlowJo v10 Software. Graphs were created using GraphPad Prism 8.

Contact us to purchase or modify this panel to meet your experimental needs

Learn how BD provides solutions to enable your COVID-19 research

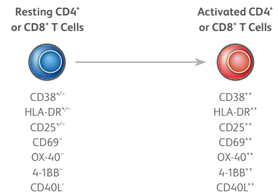
Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

Panel performance may vary when using instruments different from the one used in this experiment

These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

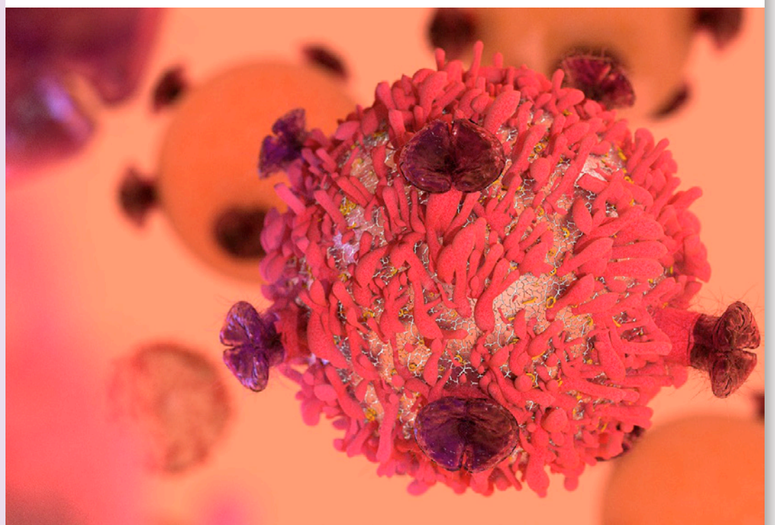
Activated T Cell Panel

The activated T cell panel is an 11-color flow cytometry panel designed for the simultaneous measurement of seven activation-induced markers (AIM) CD38, HLA-DR, CD69, CD25, CD40L, OX-40 and 4-1BB in either human CD4+ or CD8+ T cells. This panel may be used for the characterization of populations enriched for rare antigen-specific T cells, and the assessment of vaccine immunogenicity.



Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Ultraviolet 355 nm	HLA-DR	BUV395	G46-6	5 µL	564040
	CD3	BUV496	UCHT1	5 µL	612940
	CD25	BUV615	2A3	5 µL	612996
Violet 405 nm	CD137 (4-1BB)	BV421	4B4-1	5 µL	564091
	CD69	BV480	FN50	5 µL	747519
	CD8	BV750	RPA-T8	1.25 µL	747385
Blue 488 nm	CD4	FITC	RPA-T4	20 µL	555346
	Viability	7-AAD	N/A	5 µL	559925
Yellow-Green 561 nm	CD38	PE	HIT2	20 µL	555460
	CD134 (OX40)	PE-Cy7	ACT35	5 µL	563663
Red 640 nm	CD154 (CD40L)	APC	TRAP1	20 µL	555702

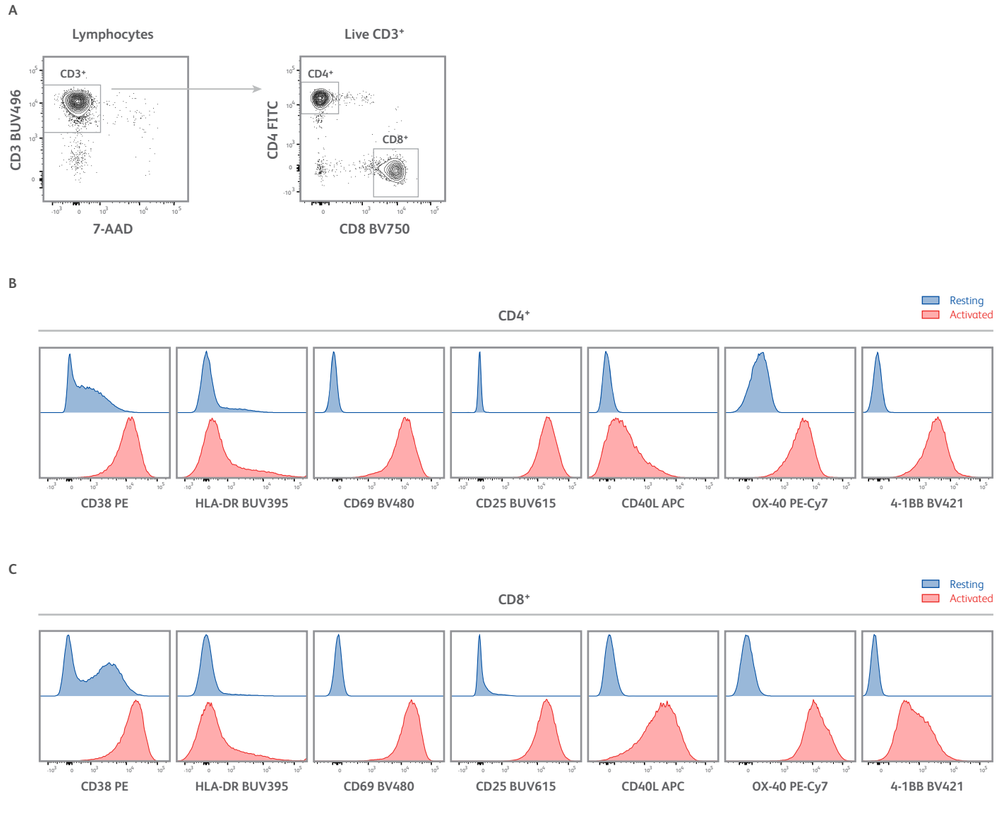


Experimental Information

Sample Type	Enriched and activated T cells
Panel Type	Multicolor panel
Protocols	PBMC isolation T cell magnetic enrichment T cell activation Surface marker staining
Panel Tested On	5-laser, 18-color (4 UV/6 violet/2 blue/3 yellow-green/3 red) BD LSRFortessa X-20 Flow Cytometer

Companion Products

Product	Application	Catalog Number
BD IMag Human T Lymphocyte Enrichment Set-DM	T cell isolation	557874
BD Horizon Brilliant Stain Buffer	Optimal stain conditions	563794



Assessment of activation-induced markers using the activated T cell panel

Representative analysis of T cells magnetically enriched from PBMCs isolated from a healthy donor. T cells were cultured for 24 hours in the absence (resting, top blue histogram) or presence (activated, bottom red histogram) of Dynabeads Human T-Activator CD3/CD28 (Thermo Fisher Scientific). **A**) Lymphocytes were first identified based on light scatter properties (not shown). Live T cells were identified as CD3+ 7-AAD- cells prior to gating of CD4+ and CD8+ T cells. **B-C**) Expression of activation markers was assessed in either resting (blue, top histogram) or activated (red, bottom histogram) CD4+ (**B**) and CD8+ (**C**) T cells. As expected, T cell activation induced upregulation of all the tested markers. The sample was acquired on a 5-laser, 18-color BD LSRFortessa X-20 Flow Cytometer. Data were analyzed using FlowJo v10 Software.

You may also be interested in the Treg backbone panel

Contact us to purchase or modify this panel to meet your experimental needs

Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

Panel performance may vary when using instruments different from the one used in this experiment

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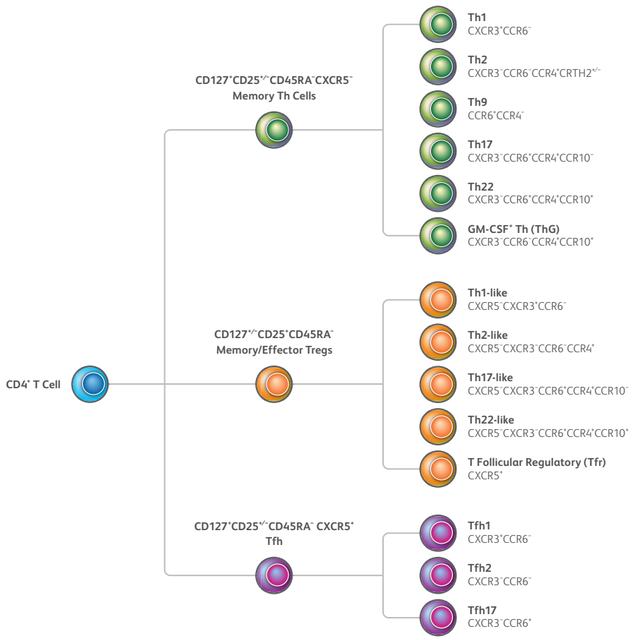
CD4+ T Cell Subset Panel

The CD4+ T cell subset panel is a 12-color flow cytometry panel designed for a comprehensive characterization of several subsets of conventional T helper cells (Th), regulatory T cells (Tregs) and T follicular helper cells (Tfh) in a single-tube assay through analysis of chemokine receptor expression patterns[1-3].



Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	CD194 (CCR4)	BV421	1G1	5 µL	562579
	CD185 (CXCR5)	BV480	RF8B2	5 µL	566142
	CD45RA	BV605	HI100	5 µL	562886
	CD127	BV711	HIL-7R-M21	5 µL	563165
	CD196 (CCR6)	BV786	11A9	5 µl	563704
Blue 488 nm	CD25	BB515	2A3	5 µL	564467
	CD294 (CRTH2)	PE	BM16	5 µL	563665
	Live/Dead	7-AAD	N/A	5 µL	559925
	CD183 (CXCR3)	PE-Cy7	1C6/CXCR3	5 µL	560831
Red 640 nm	CCR10	APC	1B5	0.15 µL*	564771
	CD3	Alexa Fluor 700	UCHT1	0.6 µL*	557943
	CD4	APC-H7	SK3	5 µL	641398

* Dilution of the stock reagent is recommended to avoid pipetting inaccuracies

1. Morita R, et al. Human blood CXCR5+CD4+ T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity* 2011;34(1):108-21. doi: 10.1016/j.immuni.2010.12.012.
2. Duhen T, et al. Functionally distinct subsets of human FOXP3+ Treg cells that phenotypically mirror effector Th cells. *Blood*. 2012;119(19):4430-4440. doi: 10.1182/blood-2011-11-392324
3. Wingender G, et al. OMIP-030: Characterization of human T cell subsets via surface markers. *Cytometry A*. 2015;87(12):1067-1069. doi: 10.1002/cyto.a.22788.

Experimental Information

Sample Type	PBMCs
Panel Type	Multicolor panel
Protocols	PBMC isolation Surface marker staining
Panel Tested On	3-laser, 12-color (5 violet/4 blue/3 red) BD FACSLyric Flow Cytometer

Companion Products

Product	Application	Catalog Number
BD Horizon Brilliant Stain Buffer	Optimal stain conditions	563794

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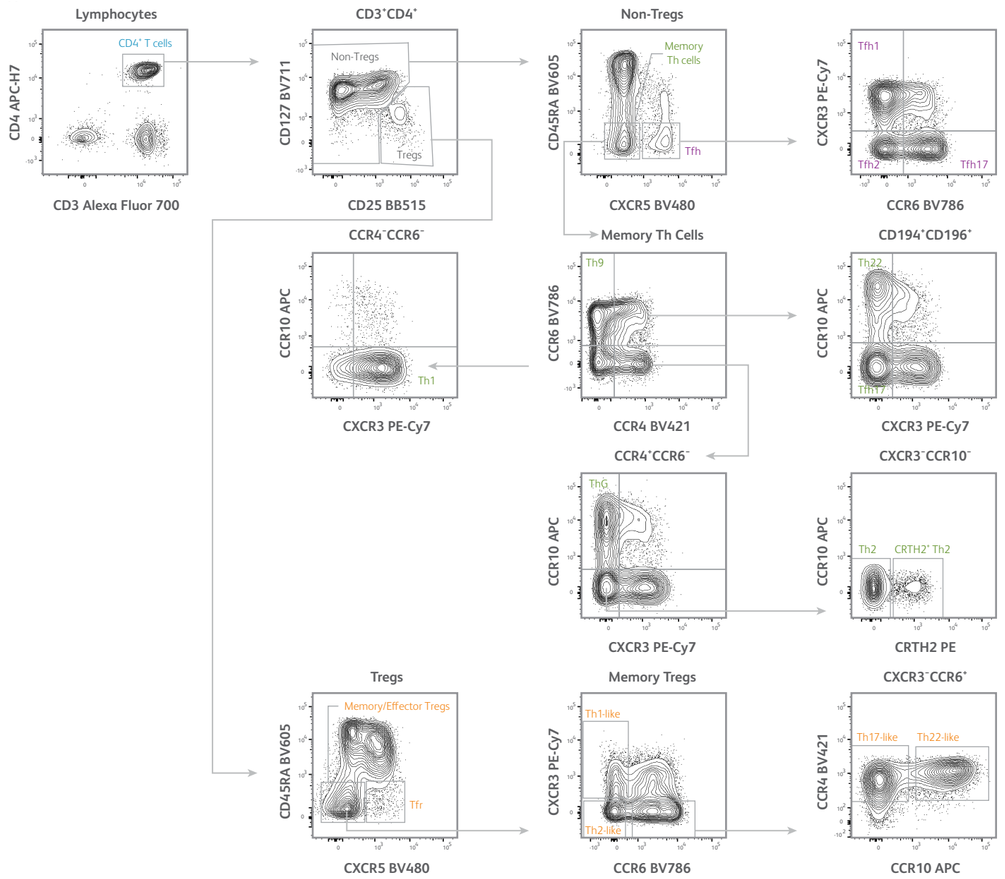

Identification of subsets of human T helper cells using the CD4+ T cell subset panel

Representative analysis of PBMCs isolated from human healthy subjects (N = 5). T cells were first gated based on scatter properties typical of lymphocytes (not shown). **A**) After gating on CD3+CD4+ T cells, regulatory T cells (Tregs) and conventional T cells (non-Tregs) could be identified based on differential expression of CD127 and CD25. From the non-Tregs gate, conventional memory Th cells and T follicular helper cells (Tfh) were defined as CD45RA-CXCR5- and CD45RA-CXCR5+ cells, respectively. Tfh could be further dissected into CXCR3+CCR6- Tfh1, CXCR3-CCR6- Tfh2 and CXCR3-CCR6+ Tfh17 subsets. From the memory Th cells gate, Th1, Th2, Th17, Th22, Th9, ThG subsets were defined based on differential expression of CXCR3, CCR4, CCR6, CCR10 and CRTH2 using a gating strategy adapted from Wingender et al.[3] **B**) From the Tregs gate, memory/effector Tregs and T follicular regulatory cells (Tfr) were defined as CD45RA-CXCR5- and CD45RA-CXCR5+ cells, respectively. Memory/effector Tregs could be further dissected into CXCR3+CCR6- Th1-like, CXCR3-CCR6- Th2-like, CXCR3-CCR6+CCR4+CCR10- Th17-like and CXCR3-CCR6+CCR4+CCR10+ Th22-like subsets. Samples were acquired on a 3-laser, 12-color BD FACSLyric Flow Cytometer. Data were analyzed using FlowJo v10 Software.

You may also be interested in the CD4+ T cell subset panel

Contact us to purchase or modify this panel to meet your experimental needs

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Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

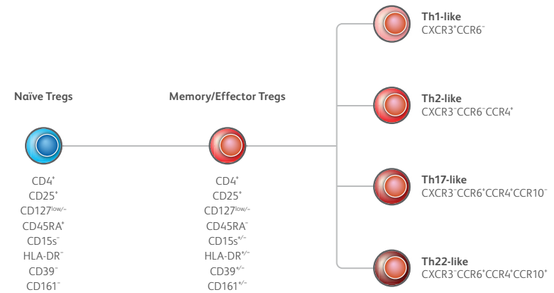
Panel performance may vary when using instruments different from the one used in this experiment

These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

Treg Backbone Panel

The Treg backbone panel is an 8-color flow cytometry panel designed for the identification of major subsets of $CD3^+CD4^+CD25^+CD127^{low/-}$ Tregs. The panel enables the characterization of naïve and memory/effector Tregs, the latter being further sub-divided in $CD15s^+$ highly differentiated Tregs and $CD161^+$ inflammatory cytokine-producing Tregs.

We show here two examples of 4-color drop-in sets for a deeper characterization of Tregs. The activated Tregs drop-ins include the activation markers PI-16, HLA-DR, CD147 and CD39. The Th-like Treg subsets drop-ins include the chemokine receptors CXCR3, CCR4, CCR6 and CCR10 for the identification of Th1-, Th2-, Th17- and Th22-like Treg subsets.



Backbone Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	CD15s	BV510	CSLEX1	5 µL	563529
	CD45RA	BV605	HI100	5 µL	562886
	CD161	BV711	DX12	5 µL	563865
	CD127	BV786	HIL-7R-M21	5 µL	563324
Blue 488 nm	Viability	7-AAD	N/A	5 µl	559925
	CD25	PE-Cy7	2A3	5 µL	335789
Red 640 nm	CD3	Alexa Fluor 700	UCHT1	0.6 µL*	557943
	CD4	APC-H7	RPA-T4	5 µL	560158

*Dilution of the stock reagent is recommended to avoid pipetting inaccuracies

Drop-Ins—Activated Tregs

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	PI-16	BV421	CRCBT-02-001	0.6 µL*	744829
Blue 488 nm	CD147	FITC	HIM6	20 µL	555962
	HLA-DR	PE	G46-6	20 µL	555812
Red 640 nm	CD39	APC	TU66	20 µL	560239

*Dilution of the stock reagent is recommended to avoid pipetting inaccuracies

Drop-Ins—Th-Like Treg Subsets

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	CD183 (CXCR3)	BV421	1C6/CXCR3	5 µL	562558
Blue 488 nm	CD196 (CCR6)	BB515	11A9	5 µL	564479
	CD194 (CCR4)	PE	1G1	5 µL	551120
Red 640 nm	CCR10	APC	1B5	0.15 µL*	564771

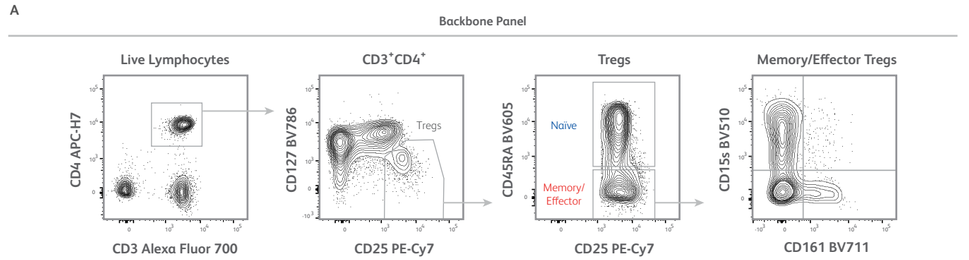
* Dilution of the stock reagent is recommended to avoid pipetting inaccuracies

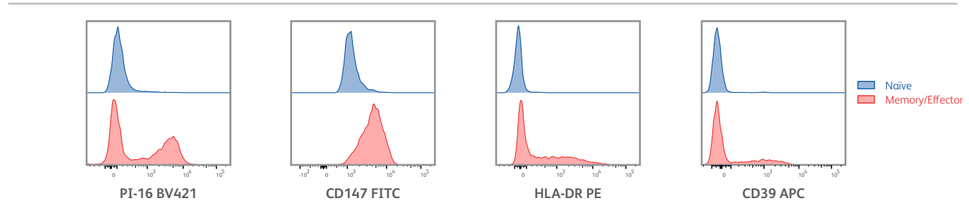
Experimental Information

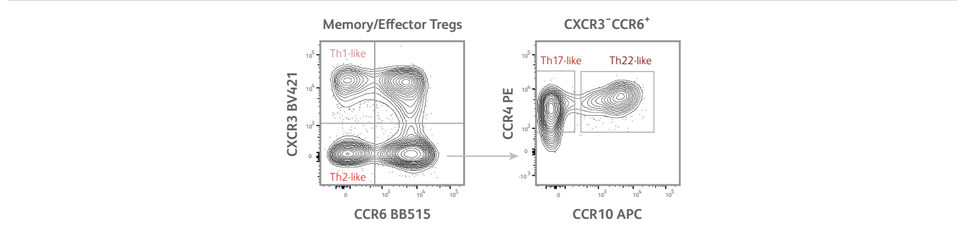
Sample Type	PBMCs
Panel Type	Backbone panel
Protocols	PBMC isolation Surface marker staining
Panel Tested On	3-laser, 12-color (5 violet/4 blue/3 red) BD FACSLyric Flow Cytometer

Companion Products

Product	Application	Catalog Number
BD Horizon Brilliant Stain Buffer	Optimal stain conditions	563794







Characterization of human regulatory T cells using the Treg backbone panel and additional drop-ins

Representative analysis of PBMCs isolated from a healthy human subject . **A**) Identification of Treg subsets using the 8-color Treg backbone panel. Live lymphocytes were first identified based on light scatter properties and 7-AAD+ dead cell exclusion (not shown). From the live lymphocyte gate, $CD3^+CD4^+$ T cells were first gated prior to identification of $CD25^+CD127^{low/-}$ Tregs. Tregs could be further divided into $CD45RA^+$ naïve and $CD45RA^-$ memory/effector Tregs. From the memory/effector Tregs gate, discrete subsets of $CD15s^+$ highly activated and $CD161^+$ inflammatory cytokine-producing Tregs were identified. **B**) Expansion of the backbone panel with four drop-ins to measure expression of markers of Treg activation (PI-16, CD147, HLA-DR, CD39). As expected, higher expression of these markers was observed in memory/effector Tregs (red, bottom histograms), as compared to naïve Tregs (blue, top histograms). **C**) Expansion of the backbone panel with four drop-ins to identify Th-like subsets of effector Tregs based on differential expression of the chemokine receptors CXCR3, CCR4, CCR6 and CCR10. Samples were acquired on a 3-laser, 12-color BD FACSLyric Flow Cytometer. Data were analyzed using FlowJo v10 Software.

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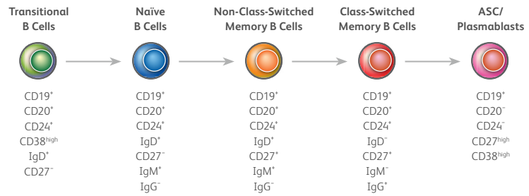
B Cell Subset Panel

The B cell subset panel is a 9-color flow cytometry panel designed to identify major subsets of B cells, including transitional, naïve, memory B cells and antibody-secreting cells (ASCs)/plasmablasts.

Two panels with same specificities, but different fluorochrome combinations, were designed for compatibility with either 3-laser (violet, blue, red) or 5-laser (UV, violet, blue, yellow-green, red) instrument configurations.



3-Laser Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	CD24	BV421	ML5	5 µL	562789
	CD27	BV480	M-T271	1.25 µL	746296
	CD19	BV605	HIB19	0.6 µL*	740394
	CD20	BV786	2H7	0.6 µL*	743611
Blue 488 nm	IgD	BB515	IA6-2	5 µL	565243
	CD38	PE	HIT2	20 µL	555460
	CD3	PerCP-Cy5.5	UCHT1	5 µL	560835
	IgG	PE-Cy7	G18-145	5 µL	561298
Red 640 nm	IgM	APC	G20-127	20 µL	551062

*Dilution of the stock reagent is recommended to avoid pipetting inaccuracies

5-Laser Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
UV Ultraviolet 355 nm	CD19	BUV395	SJ25C1	5 µL	563549
	CD3	BUV496	UCHT1	5 µL	612940
	IgD	BUV615	IA6-2	5 µL	613008
Violet 405 nm	CD24	BV421	ML5	5 µL	562789
	CD20	BV480	2H7	5 µL	566132
Blue 488 nm	CD27	BB515	M-T271	5 µL	564642
Yellow-Green 561 nm	CD38	PE	HIT2	20 µL	555460
	IgG	PE-Cy7	G18-145	5 µL	561298
Red 640 nm	IgM	APC	G20-127	20 µL	551062

Contact us to purchase or modify this panel to meet your experimental needs

Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

Panel performance may vary when using instruments different from the one used in this experiment

These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

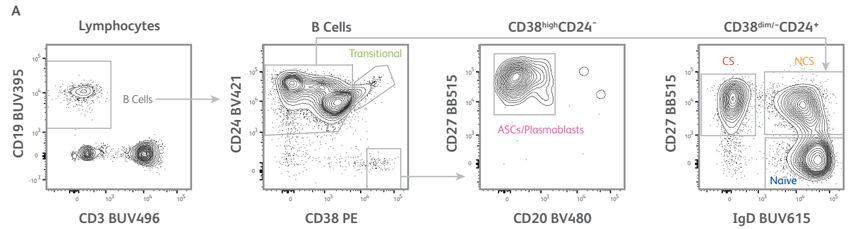
Experimental Information

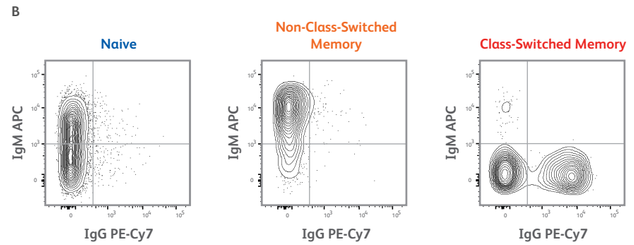
Sample Type	PBMCs
Panel Type	Multicolor panel
Protocols	PBMC isolation Surface marker staining
3-Laser Panel Tested On	3-laser, 12-color (5 violet/4 blue/3 red) BD FACSCelesta Flow Cytometer*
5-Laser Panel Tested On	5-laser, 18-color (4 UV/6 violet/2 blue/3 yellow-green/3 red) BD LSRFortessa X-20 Flow Cytometer

*Filter configuration was modified to use PE-Cy7 in place of PE-CF594

Companion Products

Product	Application	Catalog Number
BD Horizon Brilliant Stain Buffer	Optimal stain conditions	563794





Characterization of human B cells using the B cell subset panel

Representative analysis of PBMCs from healthy human donors (N = 2). Lymphocytes were first identified based on light scatter properties (not shown). **A**) From the $CD3^-CD19^+$ B cell gate, $CD38^{high}CD24^+$ transitional B cells and $CD38^{dim/-}CD24^+$ cells were identified. From the $CD38^{high}CD24^-$ gate, antibody-secreting cells (ASC) or plasmablasts were defined as $CD38^{high}CD27^{high}CD20^-$ cells. From the $CD38^{dim/-}CD24^+$ gate, naïve (blue), non-class-switched (orange) and class-switched memory (red) B cells were identified based on differential expression of CD27 and IgD. **B**) Expected expression patterns for IgM and IgG throughout B cell differentiation were observed. Samples were acquired on a 5-laser, 18-color BD LSRFortessa X-20 Flow Cytometer. Data analysis was performed using FlowJo v10 Software. A panel enabling resolution of the same B cell subsets was also designed for and tested on a 3-laser, 12-color BD FACSCelesta Flow Cytometer.

Learn more about
BD Horizon Dri Small Batch Panels

Contact us to purchase or modify this panel to meet your experimental needs

Learn how BD provides solutions to enable your COVID-19 research

Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

Panel performance may vary when using instruments different from the one used in this experiment

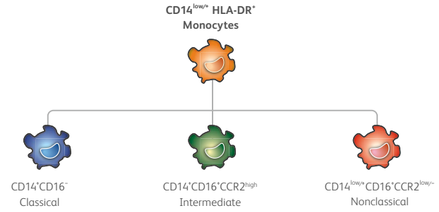
These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

BD Horizon Dri Monoset Panel

The BD Horizon Dri Monoset Panel is a 4-color flow cytometry panel designed to identify major subsets of human circulating monocytes, including classical, intermediate and nonclassical monocytes. This panel is available as unit sized, preformulated and performance-optimized dried cocktail.

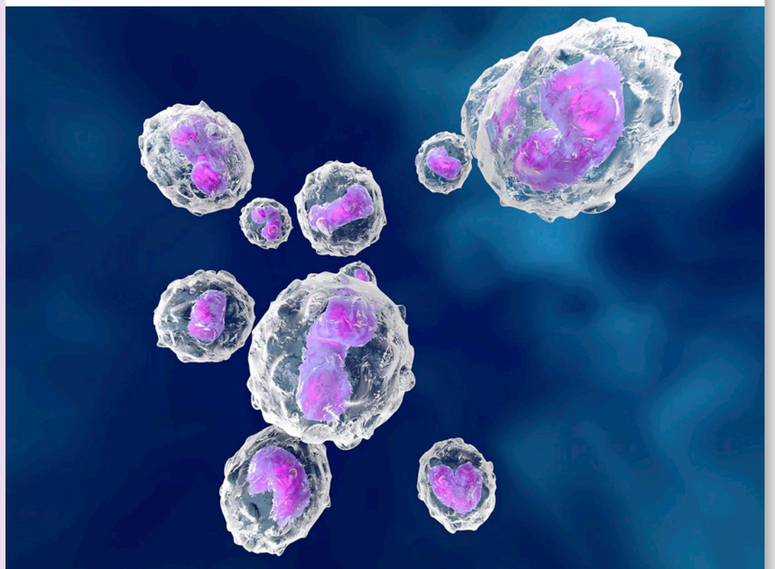
Absolute count of monocyte subsets can be measured by using BD Trucount™ Absolute Counting Tubes.

Median expression levels of HLA-DR on each monocyte subset can be calculated in antibody bound per cell (ABC) units after calibration of median fluorescence intensity (MFI) in the PE channel using BD Quantibrite™ Beads.



Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Tests Per Kit
Violet 405 nm	CD16	FITC	3G8	5
Blue 488 nm	HLA-DR	PE	L243	
	CD14	PerCP	MφP9	
Red 640 nm	CD192 (CCR2)	APC	LS132.1D9	

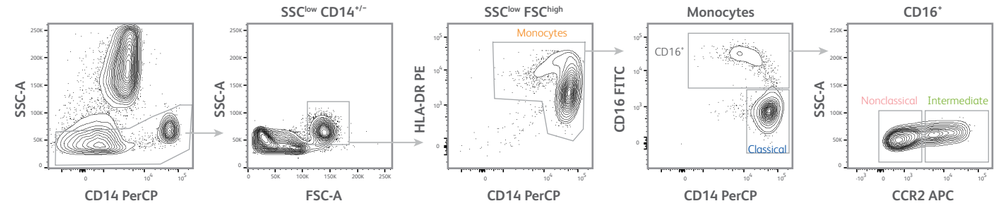


Experimental Information

Sample Type	Whole blood
Panel Type	BD Horizon Dri Small Batch
Protocols	Surface marker staining Red blood cell lysis (lyse/no wash)
Panel Tested On	3-laser, 12-color (5 violet/4 blue/3 red) BD FACSLyric Flow Cytometer

Companion Products

Product	Application	Catalog Number
BD FACS Lysing Solution	Red blood cell lysis	349202
BD Quantibrite Beads	Measurement of antigen density	340495
BD Trucount Absolute Counting Tubes	Absolute cell count	340334



Identification of subsets of human monocytes using the BD Horizon Dri Monoset Panel

Representative analysis of whole blood from healthy human subjects (N = 5). Samples were lysed with BD FACS Lysing Solution after surface marker staining using a lyse/no wash procedure. Cells were first gated as SSC^{low} CD14$^{+/-}$, followed by gating of cells with high forward scatter. From the SSC^{low}FSC^{high} gate, monocytes were identified as HLA-DR^{+} CD14$^{low/+}$ cells. From the monocytes gate, classical monocytes were identified as CD14^{+}CD16^{-} cells. From the CD16^{+}CD14$^{low/+}$ gate, CCR2^{high} intermediate and CCR2$^{low/-}$ non-classical monocytes were further detected. Samples were acquired on a 3-laser, 12-color BD FACSLyric Flow Cytometer. Data analysis was performed using FlowJo v10 Software.

Donor	Absolute Count/µL			Relative Frequency (%)			HLA-DR Expression (ABC)		
	Classical	Intermediate	Nonclassical	Classical	Intermediate	Nonclassical	Classical	Intermediate	Nonclassical
D1	469	21	33	89.7	4.0	6.2	15,750	100,025	30,884
D2	307	10	77	78.0	2.4	19.5	13,460	132,536	41,316
D3	355	29	58	80.3	6.7	13.0	16,426	99,792	38,576
D4	424	30	51	84.0	6.0	10.0	20,799	126,989	49,072
D5	341	12	37	87.4	3.0	9.5	15,502	107,601	40,860
D6	418	19	49	86.1	3.8	10.0	19,822	117,153	41,405

Monocyte subset analysis using BD Horizon Dri Monoset Panel with BD Trucount Absolute Counting Tubes and BD Quantibrite Beads. Absolute count, relative frequency and HLA-DR expression (in antibody bound per cell units) values were determined for each monocyte subset from six healthy donors.

You may also be interested in the NK cell activating and inhibitory receptor panel

Contact us to purchase or modify this panel to meet your experimental needs

Learn how BD provides solutions to enable your COVID-19 research

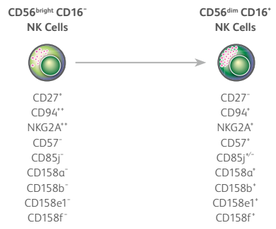
Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

Panel performance may vary when using instruments different from the one used in this experiment

These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

NK Cell Inhibitory Receptor Panel

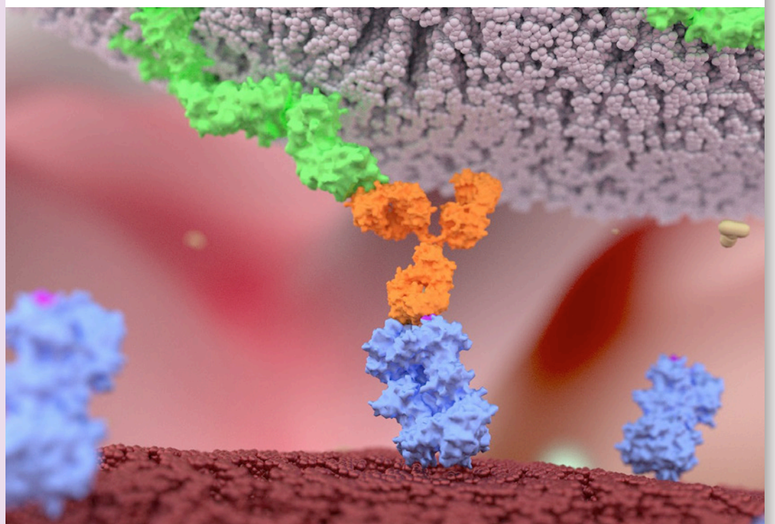
The NK cell inhibitory receptor panel is a 12-color flow cytometry panel designed for the identification of distinct subsets of NK cells based on the expression of differentiation markers CD16, CD94, CD27, CD57 and inhibitory receptors NKG2A, KIRs (CD158a, CD158b, CD158e1, CD158f) and CD85j.



Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	CD158f	BV421	UP-R1	5 µL	566330
	CD85j	BV480	GHI/75	5 µL	746434
	CD158b	BV605	CH-L	2.5 µL	743453
	CD94	BV711	HP-3D9	0.15 µL*	743952
	CD159a (NKG2A)	BV786	131411	1.25 µL	747917
Blue 488 nm	CD57	FITC	NK1	20 µL	555619
	CD158e1 (NKB1)	PE	DX9	20 µL	555967
	CD27	BB700	M-T271	5 µL	566449
	CD56	PE-Cy7	B159	5 µL	557747
Red 640 nm	CD158a	APC	HP-3E4	5 µL	564319
	CD16	Alexa Fluor 700	3G8	5 µL	557920
	CD3	APC-H7	SK7	5 µL	560176

* Dilution of the stock reagent is recommended to avoid pipetting inaccuracies

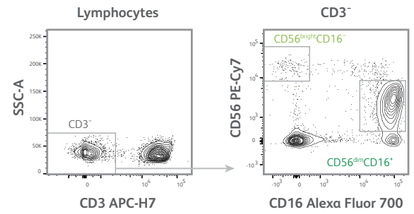


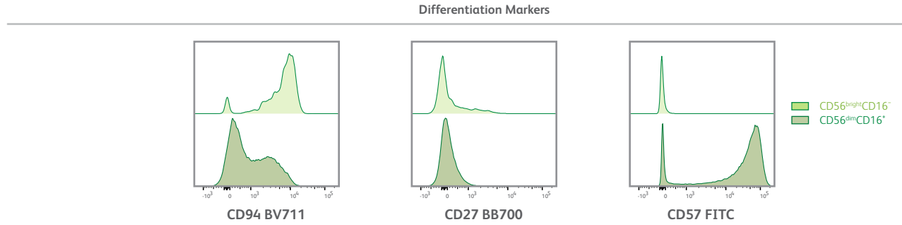
Experimental Information

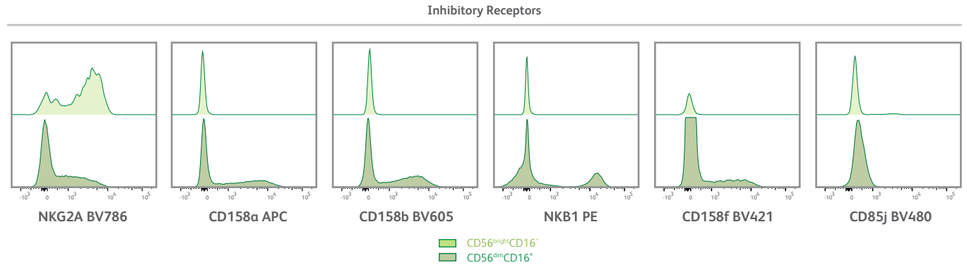
Sample Type	PBMCs
Panel Type	Multicolor panel
Protocols	PBMC isolation Surface marker staining
Panel Tested On	3-laser, 12-color (5 violet/4 blue/3 red) BD FACSLyric Flow Cytometer

Companion Products

Product	Application	Catalog Number
BD Horizon Brilliant Stain Buffer	Optimal stain conditions	563794

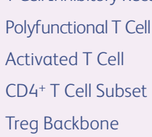






Characterization of human NK cells using the NK cell inhibitory receptor panel

Representative analysis of PBMCs isolated from a healthy human donor. Lymphocytes were first identified based on light scatter properties (not shown). $CD3^-$ non-T cells were gated prior to the identification of two major NK subsets: $CD56^{bright}CD16^-$ (light green, top histogram) and $CD56^{dim}CD16^+$ (dark green, bottom histogram) cells. The expression of differentiation markers and inhibitory receptors was assessed in the two NK cell subsets and revealed expected expression patterns. The sample was acquired on a 3-laser, 12-color BD FACSLyric Flow Cytometer. Data were analyzed using FlowJo v10 Software.



Learn how BD provides solutions to enable your COVID-19 research

You may also be interested in the NK cell inhibitory receptor panel

Contact us to purchase or modify this panel to meet your experimental needs

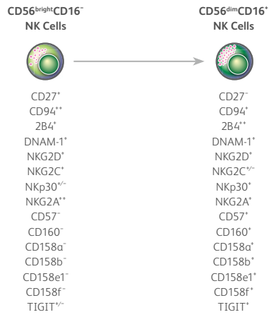
Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

Panel performance may vary when using instruments different from the one used in this experiment

These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

NK Cell Activating and Inhibitory Receptor Panel

The NK cell activating and inhibitory receptor panel is an 18-color flow cytometry panel designed for the identification of distinct subsets of NK cells based on the expression of differentiation markers CD16, CD94, CD27, CD57, activating receptors 2B4, DNAM-1, NKG2C, NKG2D, NKp30 and inhibitory receptors NKG2A, KIRs (CD158a, CD158b, CD158e1, CD158f) and TIGIT.



Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Ultraviolet 355 nm	CD158b	BUV395	CH-L	2.5 µL	743456
	CD3	BUV563	SK7	1.25 µL	741448
	CD56	BUV737	NCAM16.2	5 µL	612766
	CD158e1 (NKB1)	BUV805	DX9	0.3 µL*	748921
Violet 405 nm	CD158f	BV421	UP-R1	5 µL	566330
	CD94	BV480	HP-3D9	0.15 µL*	746737
	CD159a (NKG2A)	BV605	131411	1.25 µL	747921
	TIGIT	BV650	741182	2.5 µL	747840
	CD226 (DNAM-1)	BV711	DX11	5 µL	564796
	CD159c (NKG2C)	BV786	134591	0.3 µL*	748170
Blue 488 nm	CD57	FITC	NK-1	20 µL	555619
	CD337 (NKp30)	BB700	p30-15	2.5 µL	745937
Yellow-Green 561 nm	CD160	PE	BY55	5 µL	562118
	CD244 (2B4)	PE-CF594	2-69	5 µL	564881
	CD314 (NKG2D)	PE-Cy7	1D11	5 µL	562365
Red 640 nm	CD158a	APC	HP-3E4	5 µL	564319
	CD27	APC-R700	M-T271	5 µL	565116
	CD16	APC-H7	3G8	5 µL	560195

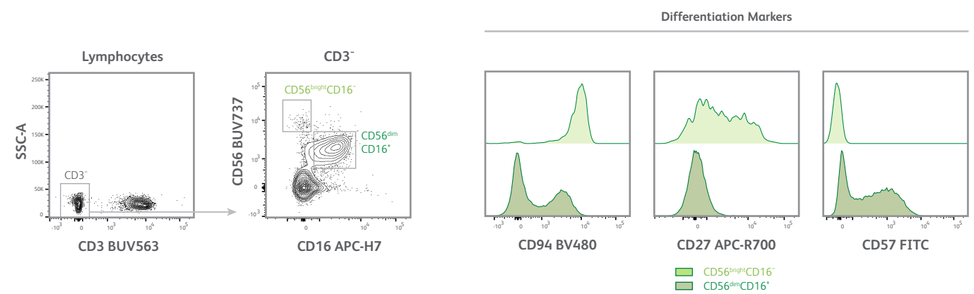
* Dilution of the stock reagent is recommended to avoid pipetting inaccuracies

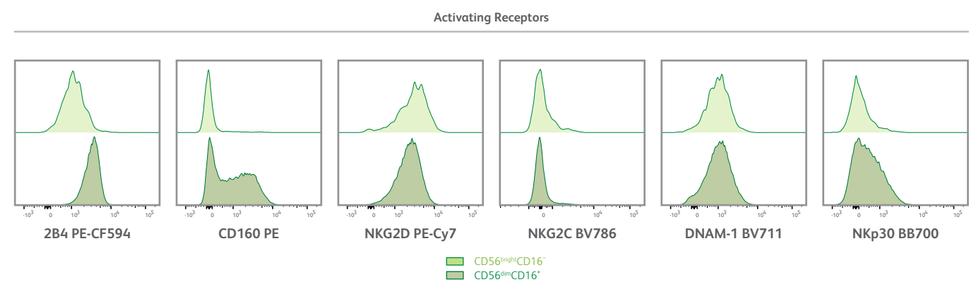
Experimental Information

Sample Type	PBMCs
Panel Type	Multicolor panel
Protocols	PBMC isolation Surface marker staining
Panel Tested On	5-laser, 18-color (4 UV/6 violet/2 blue/3 yellow-green/3 red) BD LSRFortessa X-20 Flow Cytometer

Companion Products

Product	Application	Catalog Number
BD Horizon Brilliant Stain Buffer Plus	Optimal stain conditions	566385



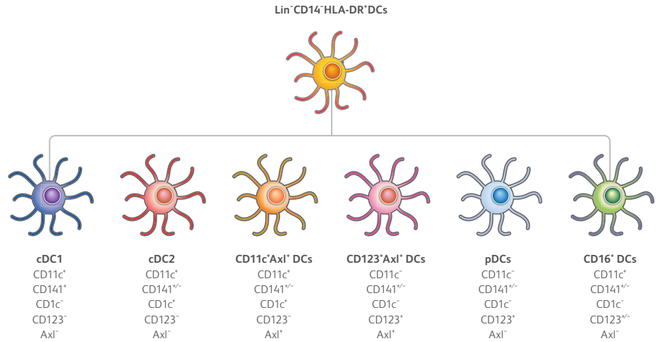


Deep immunophenotyping of human NK cells using the NK cell activating and inhibitory receptor panel

Representative analysis of PBMCs isolated from healthy human subjects (N = 5). Lymphocytes were first identified based on light scatter properties (not shown). $CD3^-$ non-T cells were gated prior to the identification of two major NK subsets: $CD56^{bright}CD16^-$ (light green, top histogram) and $CD56^{dim}CD16^+$ (dark green, bottom histogram) cells. The expression of differentiation markers, activating and inhibitory receptors was assessed in the two NK cell subsets and revealed expected expression patterns. Samples were acquired on an 18-color, 5-laser BD LSRFortessa X-20 Flow Cytometer. Data were analyzed using FlowJo v10 Software.

Dendritic Cell Subset Panel

The dendritic cell subset panel is an 18-color flow cytometry panel designed for a comprehensive characterization of human dendritic cells (DCs). This panel enables the identification of 6 major DC subsets including c DC1, cDC2, CD123⁺Axl⁺ DCs, CD11c⁺Axl⁺ DCs, pDCs and CD16⁺ DCs[1,2]. The panel further allows the analysis of eight additional surface markers used for refined DC immunophenotyping.



Contact us to purchase or modify this panel to meet your experimental needs

Optimal antibody concentrations were determined for peripheral blood cells from healthy donors and may not apply to other sample types

Panel performance may vary when using instruments different from the one used in this experiment

These reagents and panels are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

Panel Reagents

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Ultraviolet 355 nm	CD11c	BUV395	B-ly6	5 µL	563787
	CD172a/b (SIRPα)	BUV496	SE5A5	2.5 µL	749939
	CD303	BUV563	V24-785	5 µL	748415
	CD86	BUV737	2331 (FUN-1)	5 µL	612784
Violet 405 nm	CD327 (Siglec-6)	BV421	767329	2.5 µL	747915
	CD26	BV480	M-A261	1.25 µL	746696
	CD163	BV605	GHI/61	5 µL	745091
	Axl	BV650	108724	5 µL	747860
	CD36	BV750	CLB-IVC7	0.6 µL*	747253
	CD141	BV786	1A4	0.6 µL*	741006
Blue 488 nm	HLA-DR	BB515	G46-6	5 µL	564516
	CD3	BB700	SK7	5 µL	566575
	CD19	BB700	SJ25C1	5 µL	566396
	CD56	BB700	NCAM16.2	5 µL	566573
Yellow-Green 561 nm	CD370 (Clec9A)	PE	3A4/Clec9A	5 µL	563488
	CD14	PE-CF594	MφP9	5 µL	562335
	CD123	PE-Cy7	7G3	5 µL	560826
Red 637 nm	CX3CR1	Alexa Fluor 647	2A9-1	5 µL	565895
	CD1c	APC-R700	F10/21A3	5 µL	566614
	CD16	APC-H7	3G8	5 µL	560195

* Dilution of the stock reagent is recommended to avoid pipetting inaccuracies

1. Rhodes JV, et al. Human dendritic cell subsets, ontogeny, and impact on HIV infection. *Front Immunol*. 2019;10:1088. doi:10.3389/fimmu.2019.01088
2. Villani AC, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes and progenitors. *Science*. 2017;356(6335). doi:10.1126/science.aah4573.8

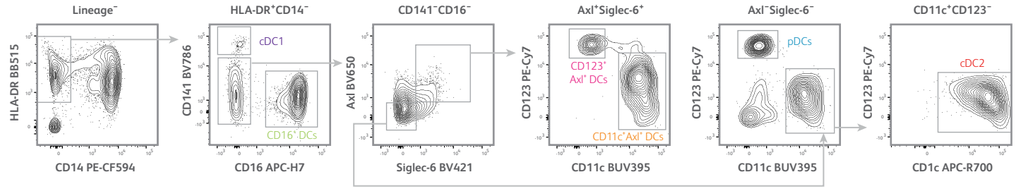
Experimental Information

Sample Type	PBMCs
Panel Type	Multicolor panel
Protocols	PBMC isolation Surface marker staining
Panel Tested On	5-laser, 28-color (7 UV/8 violet/6 blue/4 yellow-green/3 red) BD FACSymphony™ A3 Flow Cytometer

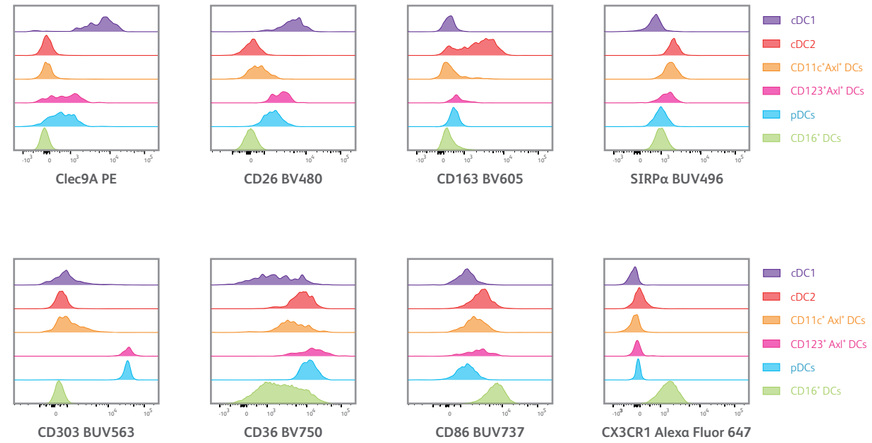
Companion Products

Product	Application	Catalog Number
BD Horizon Brilliant Stain Buffer Plus	Optimal stain conditions	566385

A



B



Deep immunophenotyping of human dendritic cells using the dendritic cell subset panel

Representative analysis of PBMCs isolated from healthy human subjects (N = 3). **A**) Identification of major dendritic cell (DC) subsets. After exclusion of CD3⁺, CD56⁺ and CD19⁺ lineage cells (not shown), DCs were identified and gated as HLA-DR⁺CD14⁻ cells. From this gate, CD141⁺ cDC1 (blue) and CD16⁺ DCs (green) were identified. From the CD141⁻CD16⁻ gate, Axl⁺ DCs were defined as Axl⁺Siglec-6⁺ cells. Axl⁺ DCs were further divided into CD123⁺ (violet) and CD11c⁺ (orange) Axl⁺ DCs. From the Axl⁻Siglec-6⁻ gate, CD123⁺CD11c⁻ pDCs (aqua) and CD11c⁺CD1c⁺ cDC2 (red) were identified. **B**) The 18-color panel further enabled the analysis of eight additional DC phenotyping markers and the identification of distinct expression patterns across the major DC subsets. Samples were acquired on a 5-laser, 28-color BD FACSymphony A3 Flow Cytometer. Data were analyzed using FlowJo v10 Software.

Panel Reagents

BD Horizon Brilliant™ Ultraviolet 395 (BUV395)	BD Horizon Brilliant Violet™ 711 (BV711)
BD OptiBuild™ Brilliant Ultraviolet 395 (BUV395)	BD Horizon Brilliant Violet™ 786 (BV786)
BD Horizon Brilliant™ Ultraviolet 496 (BUV496)	BD OptiBuild™ Brilliant Violet 786 (BV786)
BD OptiBuild™ Brilliant Ultraviolet (BUV496)	BD Pharmingen™ FITC
BD OptiBuild™ Brilliant Ultraviolet 563 (BUV563)	BD Horizon Brilliant™ Blue 515 (BB515)
BD Horizon Brilliant™ Ultraviolet 615 (BUV615)	BD Pharmingen™ PE
BD Horizon Brilliant™ Ultraviolet 737 (BUV737)	BD Horizon™ PE-CF®594
BD Horizon Brilliant™ Ultraviolet 805 (BUV805)	BD Pharmingen™ PerCP-Cy™5.5
BD Horizon Brilliant Violet™ 421 (BV421)	BD Horizon Brilliant™ Blue 700 (BB700)
BD Horizon Brilliant Violet™ 480 (BV480)	BD OptiBuild™ Brilliant Blue 700 (BB700)
BD OptiBuild™ Brilliant Violet 480 (BV480)	BD Pharmingen™ PE-Cy™7
BD Horizon Brilliant Violet™ 510 (BV510)	BD Pharmingen™ APC
BD Horizon Brilliant Violet™ 605 (BV605)	BD Pharmingen™ Alexa Fluor® 647
BD OptiBuild™ Brilliant Violet 605 (BV605)	BD Pharmingen™ Alexa Fluor® 700
BD OptiBuild™ Brilliant Violet 650 (BV650)	BD Horizon™ APC-R700
BD OptiBuild™ Brilliant Violet 750 (BV750)	BD Pharmingen™ APC-H7

Class 1 Laser Product.

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