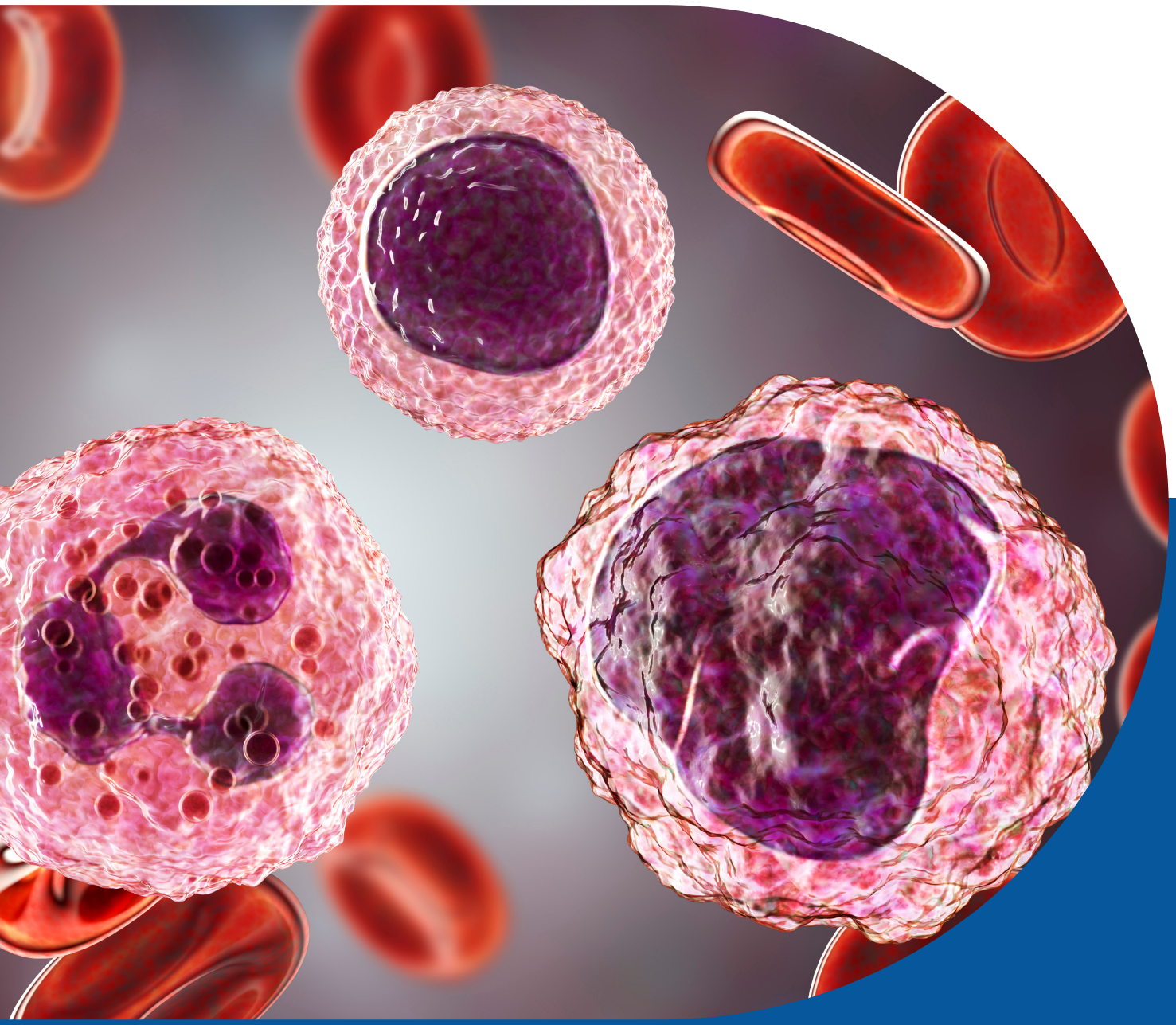


BD Leukocyte Panel Blocks

Optimised immunophenotyping panels to accelerate your high-parameter research

BD

In recent years, flow cytometry has seen a tremendous increase in the number of markers that can be run in the same panel. This opens new prospects for identifying cellular subsets, with an unprecedented level of definition. Nevertheless, increased resolution capacity goes parallel with higher experiment complexity, and the need for a careful panel design becomes pivotal.

In this eBook, we introduce pre-designed lymphoid and myeloid panel blocks that can be used as starting point for immunophenotyping. These blocks can be combined in different ways, depending on whether broader or deeper phenotypes are required. They have been designed for the conventional BD FACSymphony™ A3 and A5, but have been successfully tested on the spectral platform BD FACSymphony™ A5-SE.

By consistently using the same backbone, researchers could save time and resources, as well as gain added standardization across different projects. The different panel blocks are ready to be used for the various needs that may arise and can be implemented as projects develop.

Panel Properties

- Compatible with BD Horizon™ Fixable Viability Stain 575V
- Drop-in channels suggested with Total Spread Matrix information provided

Faster time to results

Ready-to-go backbone panels with known markers for T cells, B cells, NK cells and Innate T cells.

Standardization

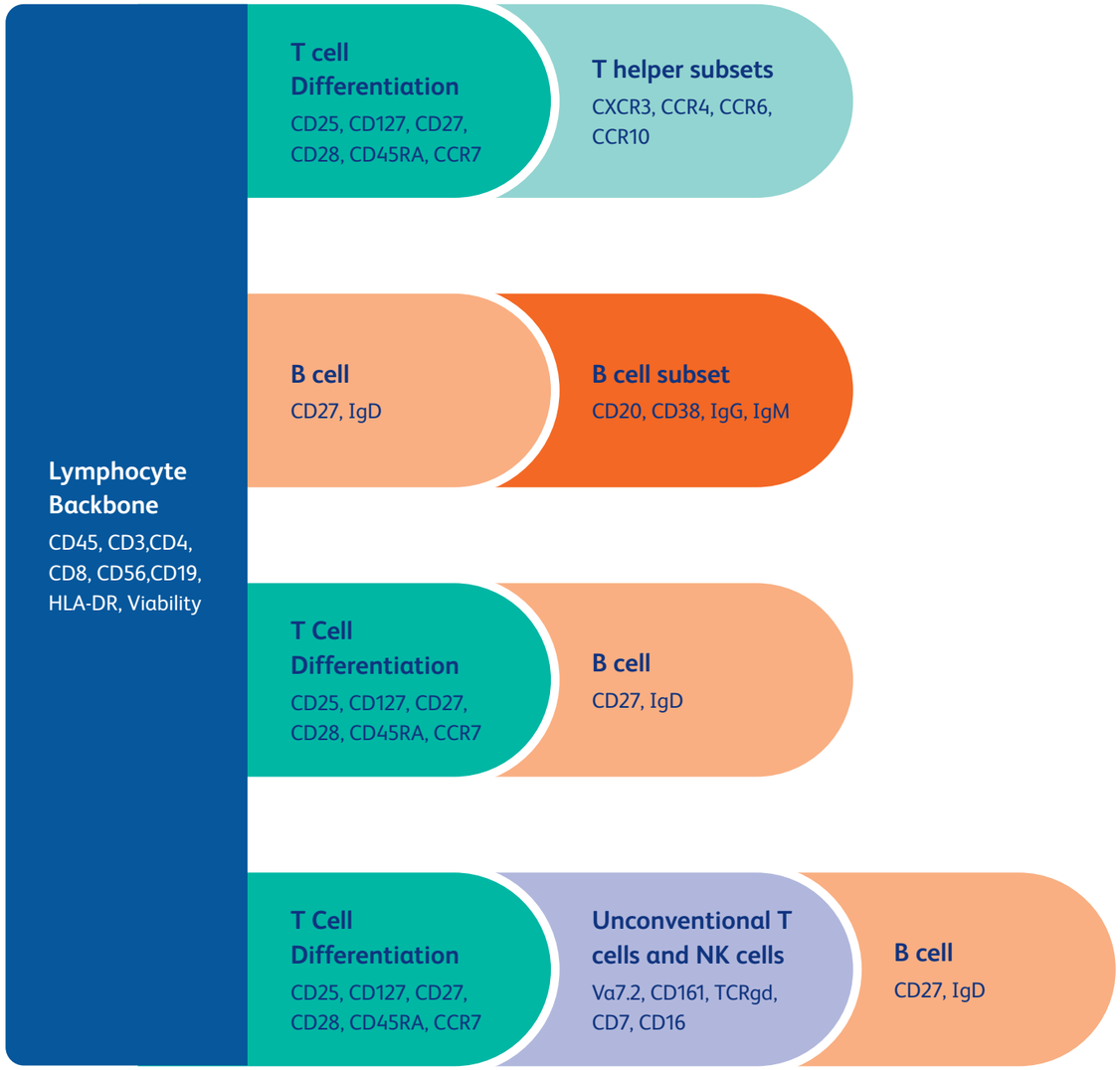
A constant set of backbone markers throughout your panels

Flexibility

The panel blocks can be combined in different ways with easy drop-in options

Lymphocyte Panel Blocks Overview

The Lymphocyte Panel Blocks consist of a shared lymphocyte backbone, in order to identify major cell subsets such as T cells, B cells, NK cells and innate T cells. This backbone can then be combined with drop-in markers allowing for deeper phenotyping.



Lymphocyte Backbone:
CD45, CD3, CD4, CD8, CD56, CD19, HLA-DR, Viability

The Lymphocyte Backbone Panel

This block consists of eight markers, including a live/dead reagent, which allows multiple T cell subsets (e.g. helper, cytotoxic, NK-T cells, activated T cells) to be identified, in addition to B cell detection.

Laser Line	Fluorochrome	Marker	Clone	Volume Per Test	Catalog Number
Ultra Violet 355 nm	BUV395	CD4	SK3 (aka Leu3a)	5 µl	563550
	BUV496	CD8	RPA-T8	5 µl	612943
	BUV563	CD19	SJ25C1 (aka SJ25-C1)	5 µl	612917
	BUV805	CD45	HI30	3 µl	612891
Violet 405 nm	FVS575V	Viability		1 µl	565694
Yellow-Green 561 nm	PE-Cy7	CD56	B159	1 µl	557747
Red 640 nm	R718	HLA-DR	L203.rMAb (aka L203)	1 µl	752501
	APC-H7	CD3	SK7 (akaLeu-4)	5 µl	560176

Table 1. Lymphocyte Backbone Panel Block. Recommended volume per test was determined using 100ul of human whole blood.

This set of backbone markers clearly resolves major lymphocyte cell subsets as described:

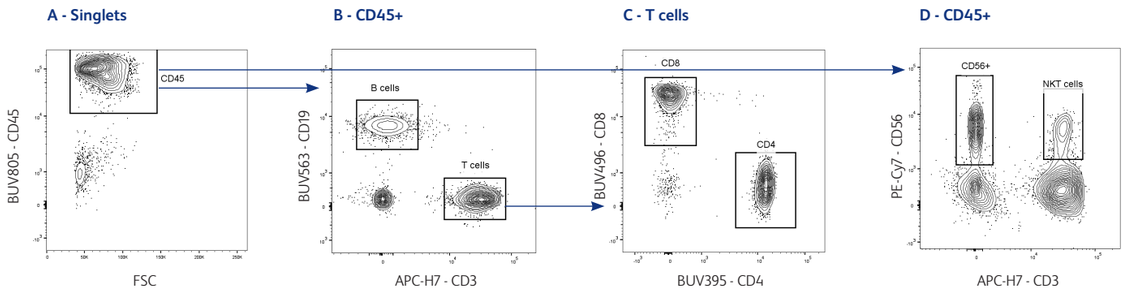


Figure 1 - Human whole blood was stained with the Lymphocyte Backbone Panel Block (Table 1) and acquired on a 5-laser BD FACSymphony™ A3 Cell Analyzer. Performance was also tested on the BD FACSymphony™ A5 and BD FACSymphony™ A5 SE Cell Analyzers. Initial gating was done on the lymphocyte population. Plot A is gated from singlets. Clear resolution of all cell subsets was observed irrespective of the instrument used (not shown).

Introduction | Lymphocyte Panel Blocks | Myeloid Panel Blocks | Protocols

The Total Spread Matrix

While designing multicolor flow cytometry panels, it is important to assess fluorescence spread to prevent or minimize loss of resolution. The Spillover Spread Matrix (SSM) was initially developed as a tool to monitor and compare instrument performance over time, especially when experiments are standardized or calibrated across different instruments.[1]

The SSM is independent of fluorochrome brightness. While this feature is important for the comparison of instruments, it may lead to inaccurate spread prediction and sub-optimal panel design. For this reason, the Total Spread Matrix (TSM) was developed as the Spillover Spreading Matrix without normalization to the fluorescent intensity of the probe. This makes it possible to evaluate the true spreading in a given panel based on the signal intensity of the different marker/fluorochrome combinations in each channel. This tool was used to develop all of the panels shown in this eBook.
As shown in the example below, spread into the additional channels of the Lymhocyte Backbone Panel is kept to a minimum, facilitating an easy addition of markers. SSM and TSM can be calculated using appropriate samples with FlowJo™ as of version 10.7.2.

Click here or scan the QR code to find out more about this new, optimized tool for more accurate spread assessment, and to also view the TSM matrices and instrument configuration for all of the panel blocks collected on the BD FACSymphony™ A3.

Total Spread Matrix

		BUV 615	BUV 661	BUV 737	BV 421	BV 480	BV 605	BV 650	BV 711	BV 750	BV 786	BB 515	BB 630	BB 660	BB 700	RB 744	RB 780	PE	PE-CF594	PE-Cy5	APC
Total Spread From Backbone Markers	BUV395 CD4	11	21	21	0	0	60	24	13	40	26	0	13	21	17	22	29	29	26	18	22
	BUV496 CD8	15	56	43	0	78	94	36	25	57	40	18	10	24	29	22	39	55	55	0	48
	BUV563 CD19	18	53	35	0	0	72	0	8	23	0	0	38	35	17	22	10	**169**	120	48	45
	BUV805 CD45	13	36	62	31	17	65	29	20	121	118	21	23	22	22	48	113	38	38	15	31
	PE-Cy7 CD56	12	22	14	17	0	0	10	18	82	41	0	0	16	28	148	**284**	28	40	16	18
	R718 HLA-DR	0	22	**230**	0	0	22	22	44	**218**	92	0	1	37	34	56	116	15	0	46	128
	APC-H7 CD3	9	26	36	0	16	57	25	22	140	123	24	22	25	22	48	140	24	32	29	44

Table 2. The Total Spread Matrix showing spread from markers in the Lymphocyte Backbone Panel Block. The channels with the highest spread are highlighted.

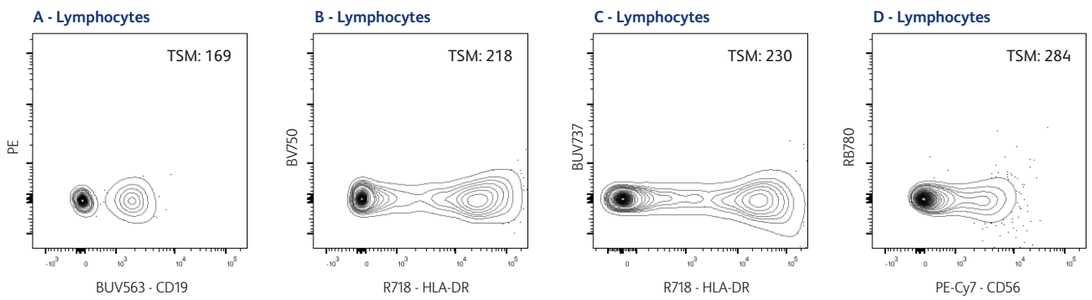


Figure 2 - Representative data for TSM values in Table 2. Human whole blood was stained with the Lymphocyte Backbone Panel and TSM values calculated in FlowJo v10.8.1 Software. Initial gating was done on the lymphocyte population.

T cell Differentiation:
CD25, CD127, CD27, CD28, CD45RA, CCR7

The T Cell Differentiation Panel Block

The T Cell Differentiation Panel Block consists of six markers, describing the Treg subset (CD25 and CD127) and full T cell maturation (CD45RA, CCR7, CD27, CD28).[2]

Laser Line	Fluorochrome	Marker	Clone	Volume Per Test	Catalog Number
Violet 405 nm	BV605	CD127	HIL-7R-M21	5 µl	562662
	BV650	CD27	L128	5 µl	563228
	BV786	CD28	L293	5 µl	742530
Blue 488 nm	BB515	CD45RA	HI100	5 µl	564552
Yellow-Green 561 nm	PE-CF594	CD25	M-A251	5 µl	562403
Red 640 nm	APC	CCR7	2-L1-A	2 µl	566762

Table 3. T Cell Differentiation Panel Block. Recommended volume per test was determined using 100ul of human whole blood.

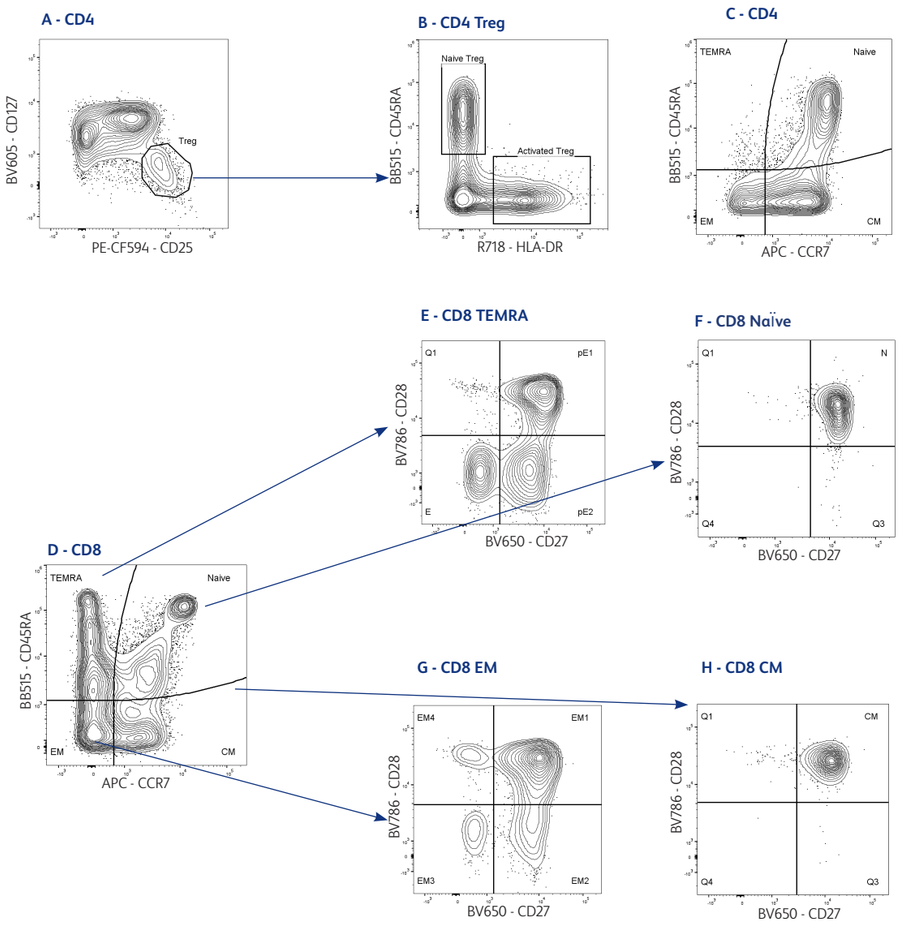


Figure 3 - Human whole blood was stained with the Lymphocyte Backbone Panel Block (Table 1) and The T Cell Differentiation Panel Block (Table 3) and acquired on a 5-laser BD FACSymphony™ A3 Cell Analyzer. Performance was also tested on the BD FACSymphony™ A5 and BD FACSymphony™ A5 SE Cell Analyzers. Clear resolution of the T cell subsets was observed irrespective of the instrument utilized (not shown). Initial gating was done according to the gating strategy for the Lymphocyte Backbone Panel Block after which, Treg and CD4 maturation was gated from the CD4 gate and CD8 maturation from the CD8 gate.

Expanding the panels by using drop-ins

The BD Leukocyte Panel Blocks are designed to be used together and reduce the need for extensive panel design effort. For that reason, the relative brightness of possible drop-ins are shown below to ensure resolution and minimize issues with spread.

	Very Bright	Bright	Moderate	Dim
Ultraviolet 355 nm		BD Horizon™ BUV563 BD Horizon™ BUV615 BD Horizon™ BUV661 BD Horizon™ BUV737	BD Horizon™ BUV395 BD Horizon™ BUV496	BD Horizon™ BUV805
Violet 405 nm	BD Horizon™ BV421 BD Horizon™ BV650 BD Horizon™ BV711	BD Horizon™ BV480 BD Horizon™ BV605 BD Horizon™ BV786	BD Horizon™ BV510 BD Horizon™ BV750	BD Horizon™ V450 BD Horizon™ V500
Blue 488 nm	BD Horizon™ BB515 BD Horizon™ BB700 BD Horizon™ PE-CF594 PE-Cy5 BD Horizon™ RB545 BD Horizon™ RB705 BD Horizon™ RB744 BD Horizon™ RB780	PE PE-Cy7	FITC Alexa Fluor™ 488 PerCP-Cy5.5	PerCP
Yellow/ Green 561 nm	PE BD Horizon™ PE-CF594 PE-Cy5 PE-Cy7 BD Horizon™ RY586			
Red 640 nm		APC Alexa Fluor™ 647 BD Horizon™ APC-R700 BD Horizon™ R718		Alexa Fluor™ 700 APC-H7 APC-Cy7

Table 4. Relative Brightness of Recommended Drop-ins[3]

Drop-ins can be placed in any empty channel, depending on the panel blocks used.

When adding new markers, make sure to adhere to the panel design guidelines and even consult instrument-specific spreading matrixes. For more information on basic panel design guidelines please visit:

www.bdbiosciences.com/en-eu/resources/panel-design#Overview

tips&tricks

Pre-Incubate with antibodies against chemokine receptors at 37°C for 10 min to increase resolution of chemokine receptors.

Chemokine receptor staining is extremely susceptible to sample quality and handling, e.g. temperature. If cells have been frozen, it is beneficial to incubate them at 37°C over night.

Sign up for a free BD Research Cloud account to explore these panel blocks, and easily add drop-in reagents for your experiment.

The T Helper Cell Subset Panel Block

This panel block consists of chemokine receptor markers identifying polarization of activated T helper cells into Th1, Th2, and Th17 subsets.[4]

Laser Line	Fluorochrome	Marker	Clone	Volume Per Test	Catalog Number
Ultra-Violet 355 nm	BUV661	CXCR3	1C6/CXCR3 (aka 1C6, LS177-1C6)	5 µl	741649
	BUV737	CCR6	11A9	1 µl	612780
Violet 405 nm	BV421	CCR4	1G1	5 µl	562579
Yellow-Green 561 nm	PE	CCR10	1B5	1 µl	563656

Table 5. T Helper Cell Subset Panel Block. Recommended volume per test was determined using 100ul of human whole blood.

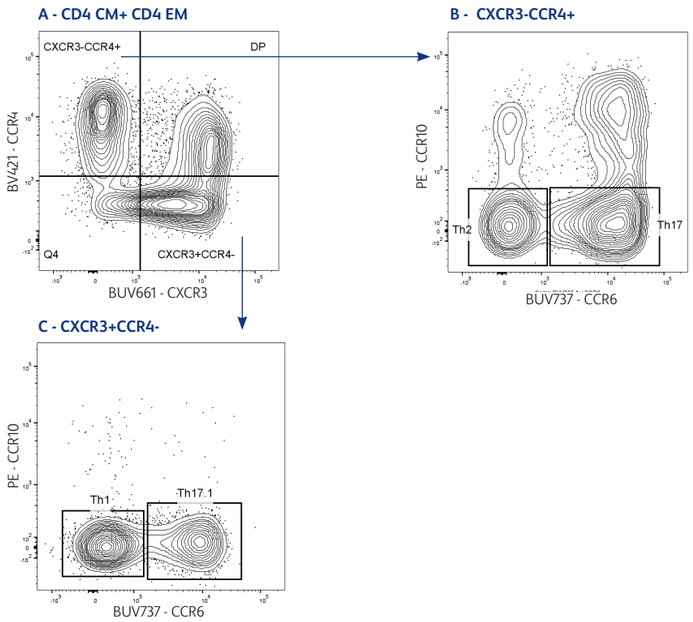


Figure 4 - Human whole blood was stained with the Lymphocyte Backbone Panel Block (Table 1) and The T Cell Differentiation Panel Block (Table 3) and T Helper Cell Subset Panel Block (Table 5) and acquired on a 5-laser BD FACSymphony™ A3 Cell Analyzer. Performance was also tested on the BD FACSymphony™ A5 and BD FACSymphony™ A5 SE Cell Analyzers. Clear resolution of the T cell subsets was observed irrespective of the instrument utilized (not shown). T cells were gated as shown for the Lymphocyte Backbone Panel Block and memory cells identified with the T Cell Differentiation Panel Block. The chemokine receptor expression determined the CD4 EM and CM compartments (see gating for the T Cell Differentiation Panel Block).

Drop-ins for T Stem Cell Central Memory Cells (SCM) and T Precursor exhausted cells using spectral flow cytometry

T stem central memory cells (SCM) are a rare subset of memory lymphocytes showing stem cell-like features and the capacity to reconstitute the spectrum of memory and effector subsets. They have naïve phenotype CD45RA$^+$, CD45RO$^-$, CCR7$^+$, CD27$^+$, being positive for CD95 and negative for the exhaustion markers.

T precursor exhausted (T-pex) cells are a specialized population of antigen experienced CD8$^+$ T lymphocytes showing features of exhausted and early memory cells. This population can be characterized by flow cytometry being positive for CCR7, CD95, CD27 and bearing the expression of TGIT and PD-1. The subset is shown to be committed to generate dysfunctional exhausted like progeny, and it has been described to undergo proliferation generating effector T cells in response to immune checkpoint blockade using anti PD-1 drugs.

T stem cells, share the T-pex phenotype, with the exception of exhaustion marker positivity, and give origin to fully functional progeny.[5,6]

The below plots show CD8$^+$ naïve, T-pex SCM and CM gating strategy for the markers shown in table 6, following the spectral implementation of the lymphocyte panel and the data acquisition BD FACSymphony™ A5 SE Cell Analyzers platform.

Laser Line	Fluorochrome	Marker	Clone	Volume Per Test	Catalog Number
Violet 405 nm	BV480	CD45RO	UCHL1	5 µl	566143
	BV711	CD95	DX2	5 µl	563132
Blue 488 nm	RB545	CD16	3G8	5 µl	569243
	RB780	TIGIT	741182	1 µl	755561
Yellow-Green 561 nm	RY586	PD-1	EH12.1	5 µl	568118

Table 6. Drop-in Markers for SCM analysis. Recommended volume per test was determined using 100ul of human whole blood.

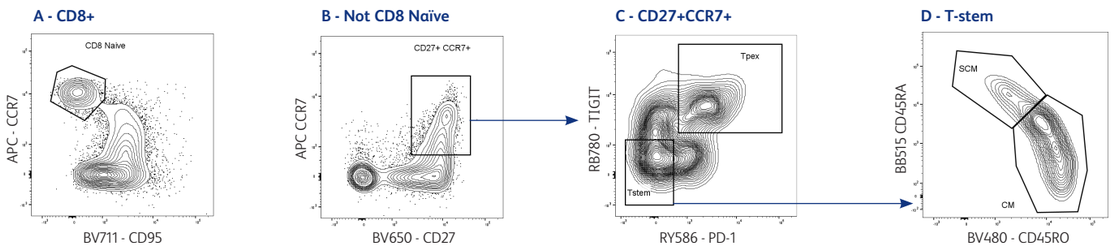


Figure 5 - Human whole blood was stained with the Lymphocyte Backbone Panel Block (Table 1), the T cell Differentiation Panel Block (Table 3), the T Helper Cell Subset Panel Block (Table 5) and additional drop-in markers (Table 6) and acquired on a BD FACSymphony™ A5 SE Cell Analyzer. Tpex, Tstem and CM, SCM cells were identified by gating on CD8$^+$ T cells as shown for the Lymphocyte Backbone Panel Block and further gated as shown above. Resolution of other key subsets in the panels was not affected by these additional markers (not shown).

B cell:
CD27, IgD

B cell subset:
CD20, CD38, IgG, IgM

The B Cell Maturation and Subset Panel Blocks

These two panels allow comprehensive B cell phenotyping.[7] CD27 and IgD can be used to describe the CD19⁺ cell maturation curve. CD20, CD27, and CD38 allow bona fide discrimination of plasma cells and transitional cells. IgD, IgG, and IgM can identify the process of isotype switching along maturation.

Laser Line	Fluorochrome	Marker	Clone	Volume Per Test	Catalog Number
Violet 405 nm	BV480	IgD	IA6-2 (aka δ-IA6-2)	5 µl	566138
	BV650	CD27	L128	5 µl	563228

Table 7. B Cell Panel Block. Recommended volume per test was determined using 100ul of human whole blood.

Laser Line	Fluorochrome	Marker	Clone	Volume Per Test	Catalog Number
Ultra-Violet 355 nm	BUV661	IgG	G18-145	5 µl	741639
	BUV737	CD20	2H7	5 µl	612849
Violet 405 nm	BV786	CD38	HIT2	5 µl	563964
Yellow-Green 561 nm	PE-CF594	IgM	G20-127	5 µl	562539

Table 8. B Cell Subset Panel Block. Recommended volume per test was determined using 100ul of human whole blood.

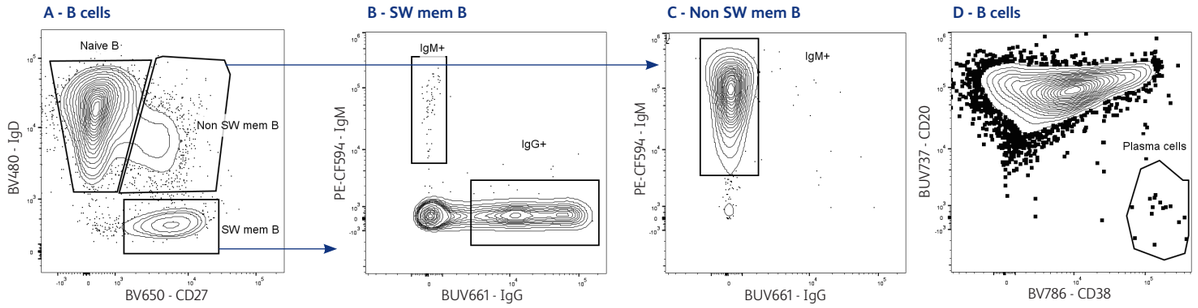


Figure 6 - Washed human whole blood was stained with the Lymphocyte Backbone Panel Block (Table 1) The B cell Panel Block (Table 7) and B Cell Subset Panel Block (Table 8) and acquired on a 5-laser BD FACSymphony™ A3 Cell Analyzer. Performance was also tested on the BD FACSymphony™ A5 and BD FACSymphony™ A5 SE Cell Analyzers. Clear resolution of the B cell subsets was observed irrespective of the instrument utilized (not shown). B cells were gated according to the Lymphocyte Backbone Panel Block. Furthermore, performance of the B Cell Panel Block was tested both with the B Cell Subset Panel Block as well as the T Cell Subset Panel Block with comparable results.

Unconventional T cells and NK cells:
Vα7.2, CD161, TCRgd, CD7, CD16

Unconventional T Cells and NK Cell Panel Block

This panel block is dedicated to NK and unconventional T cell phenotyping. γδ T cells are captured by the corresponding antibody. Mucosal Associated Invariant T cells (MAIT) are phenotyped as CD161⁺ and TCR-Vα 7.2⁺, and NKT cells are classically defined as CD3⁺ CD56⁺.[8,9]

Laser Line	Fluorochrome	Marker	Clone	Volume Per Test	Catalog Number
Ultra-Violet 355 nm	BUV615	TCRgd	11F2	5 µl	751308
	BUV737	CD16	3G8	0.5 µl	612786
Violet 405 nm	BV711	CD161	DX12	10 µl	563865
	BV750	CD7	M-T701	5 µl	747209
Blue 488 nm	BB700	TCR-Vα 7.2	OF-5A12	5 µl	749483

Table 9. Unconventional T Cells and NK Cell Panel Block. Recommended volume per test was determined using 100ul of human whole blood.

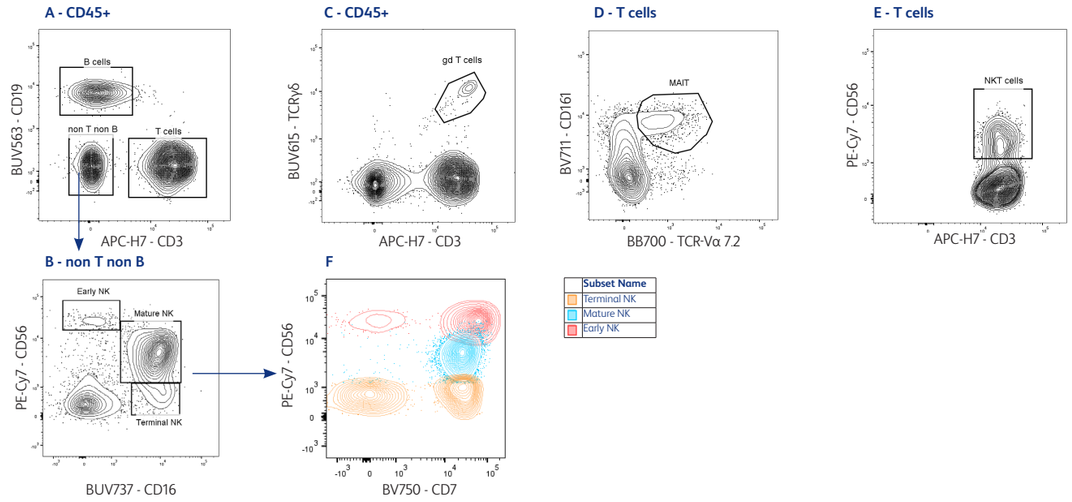


Figure 7 - Human whole blood was stained with the Lymphocyte Backbone Panel Block (Table 1) and the Unconventional T Cells and NK Cell Panel Block (Table 9) and acquired on a 5-laser BD FACSymphony™ A3 Cell Analyzer. Performance was also tested on the BD FACSymphony™ A5 and BD FACSymphony™ A5 SE Cell Analyzers. Clear resolution of the cell subsets was observed irrespective of the instrument utilized (not shown). MAIT and γδT cells were analyzed by first gating on T cells from the Lymphocyte Backbone Panel Block whereas the NK cells were gated straight from the CD45+ (see the Lymphocyte Panel Block).

The Unconventional T Cells and NK Cell Panel Block can be used together with the T Cell and B Cell Primary Panel Blocks, creating a 20 color TBNK panel.

On the other hand, this block can also be used alone with only the Lymphocyte Backbone Panel Block. This gives room for plenty of drop-in channels, with very low pre-existing spread for a deeper phenotyping (see Total Spread Matrix).

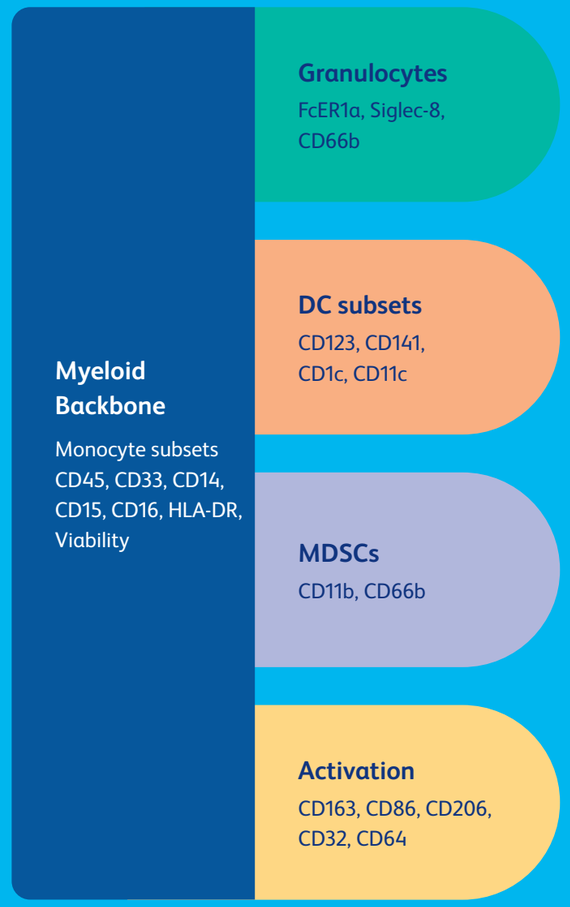
Myeloid Panel Blocks Overview

The Myeloid Panel Blocks consist of a shared myeloid backbone including the generic myeloid marker, CD33, and further monocyte markers that act as a base for the other phenotypic blocks.

These blocks allow deeper phenotyping of Dendritic Cells, Granulocytes, Myeloid Derived Suppressor Cell, including the evaluation of some activation markers.

The backbone and blocks can be used all together or in the preferred combinations.

Given the differential marker expression within the myeloid compartment, the markers in this panel can be used in a wide variety of combinations.



Myeloid Backbone:
CD45, CD33, CD14, CD15, CD16, HLA-DR, Viability

tips&tricks

Use the new-and-improved **BD Spectrum Viewer** to determine appropriate filters, as well as fluorochrome compatibility and fluorescent spillover.

tips&tricks

Using a lineage exclusion for lymphocytes will increase accuracy in subset determination and increase resolution.

The Myeloid Backbone Panel

The Myeloid Panel Block consists of a backbone panel which can be implemented in a variety of ways, with four different deep-dive panels to phenotype: granulocytes, DCs, Myeloid-derived Suppressor Cells (MDSC) and explore additional myeloid markers for further subsetting.[10]

Laser Line	Fluoro-chrome	Marker	Clone	Volume Per Test	Catalog Number
Ultra-Violet 355 nm	BUV496	CD16	3G8	1 µl	612944
	BUV563	Lineage dump			
		CD3 CD19			
	BUV805	CD45	HI30	3 µl	612891
Violet 405 nm	FVS575	Viability			565694
	BV786	CD15	HI98 (also known as HIM1)	1 µl	563838
Blue 488 nm	BB515	CD33	WM53 (also known as WM-53)	1 µl	564588
Red 640 nm	Alexa Fluor™ 700	HLA-DR	G46-6	1 µl	560743
	APC-H7	CD14	MφP9	1 µl	560180

Table 10 . Myeloid Backbone Panel Block. Recommended volume per test was determined using 100ul of human whole blood.

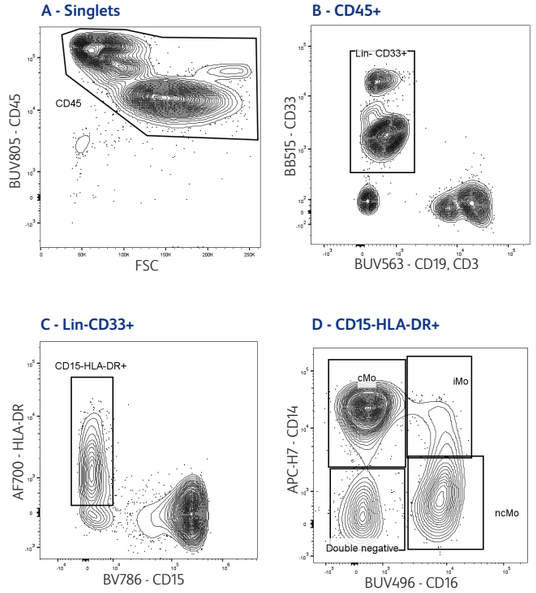


Figure 8 - Human whole blood was stained with the Myeloid Backbone Panel Block (Table 10) and acquired on a 5-laser BD FACSymphony™ A3 Cell Analyzer. Performance was also tested on the BD FACSymphony™ A5 and BD FACSymphony™ A5 SE Cell Analyzers. Clear resolution of the cell subsets was observed irrespective of the instrument utilized (not shown). Gating is done directly from the singlet gate. Cells that are Lin-, CD33+ HLA-DR+, CD14-CD16 double negative can be further analyzed with dendritic cell markers. Classical Monocytes (cMO), intermediate Monocytes (iMo), non-classical monocytes (ncMo).

The Dendritic Cell (DC) Subset Panel Block

The four markers included here allow the discrimination of plasmacitoyd Dendritic Cells p(DC) via CD11c$^-$ and CD123$^+$, Conventional DC1 (cDC1) via CD11c$^+$ and CD141$^+$, and Conventional DC2 (cDC2) via CD11c$^+$ and CD1a$^+$.[11]

Laser Line	Fluorochrome	Marker	Clone	Volume Per Test	Catalog Number
Ultra-Violet 355 nm	BUV615	CD141	1A4	5 µl	752356
Violet 405 nm	BV480	CD11c	B-ly6	5 µl	566135
Blue 488 nm	BB700	CD1c	F10/21A3	1 µl	746095
Yellow-Green 561 nm	PE-Cy7	CD123	7G3	1 µl	560826

Table 11. DC Subset Panel Block. Recommended volume per test was determined using 100ul of human whole blood.

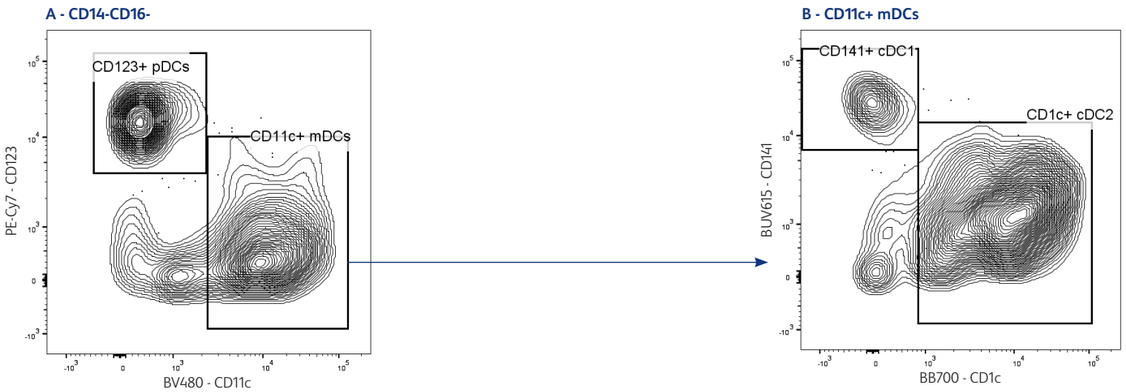


Figure 9 - Human whole blood was stained with the Myeloid Backbone Panel Block (Table 10) and the DC Subset Panel Block (Table 11) and acquired on a 5-laser BD FACSymphony™ A3 Cell Analyzer. Performance was also tested on the BD FACSymphony™ A5 and BD FACSymphony™ A5 SE Cell Analyzers. Clear resolution of the cell subsets was observed irrespective of the instrument used (not shown). Initial gating was done according to the Myeloid Backbone Panel Block, gating on CD45+, Lin-, non-basophils, HLA-DR+, and CD14-CD16- (see Myeloid Backbone Panel Block). Plasmacytoid DCs (pDCs), myeloid DCs (mDCs) and conventional DCs (cDCs).

The Granulocyte Panel Block

This panel block enables characterization of the different subpopulations of granulocytes, where eosinophils and neutrophils can initially be gated by high SSC and CD15 expression, and basophils will be found in the SSC low CD15$^-$ cell fraction. Further characterization using a combination of this panel block, as well as the backbone markers CD16 and HLA-DR will further determine each subtype.[12,13,14]

Laser Line	Fluorochrome	Marker	Clone	Volume Per Test	Catalog Number
Violet 405 nm	BV605	FcER1a	AER-37 (aka CRA-1, CRA1)	2 µl	747785
	BV711	Siglec 8	837535	1.5 µl	747870
Red 640nm	Alexa Fluor™ 647	CD66b	G10F5	1 µl	561645

Table 12. Granulocyte Panel Block. Recommended volume per test was determined using 100ul of human whole blood.

Figure 10 - Human whole blood was stained with the Myeloid Backbone Panel Block (Table 10) and the Granulocyte Panel Block (Table 12) and acquired on a 5-laser BD FACSymphony™ A3 Cell Analyzer. Performance was also tested on the BD FACSymphony™ A5 and BD FACSymphony™ A5 SE Cell Analyzers. Clear resolution of the cell subsets was observed irrespective of the instrument utilized (not shown). Initial gating was done according to the Myeloid Backbone Panel Block, gating on CD45pos (see Myeloid Backbone Panel Block).

Myeloid-derived Suppressor Cell (MDSC) Panel Block

There are two major groups of MDSCs, either derived from the polymporphonuclear - PMN-MDSCs or the monocytic M-MDSCs compartment. The three markers in this panel, together with HLA-DR are considered the minimum for identifying these cells.[15]

Laser Line	Fluorochrome	Marker	Clone	Volume Per Test	Catalog Number
Ultra-Violet 355 nm	BUV395	CD11b	ICRF44 (also known as 44)	1 µl	563839
Red 640 nm	Alexa Fluor™ 647	CD66b	G10F5	1 µl	561645

Table 13. MDSC Panel Block. Recommended volume per test was determined using 100ul of human whole blood.

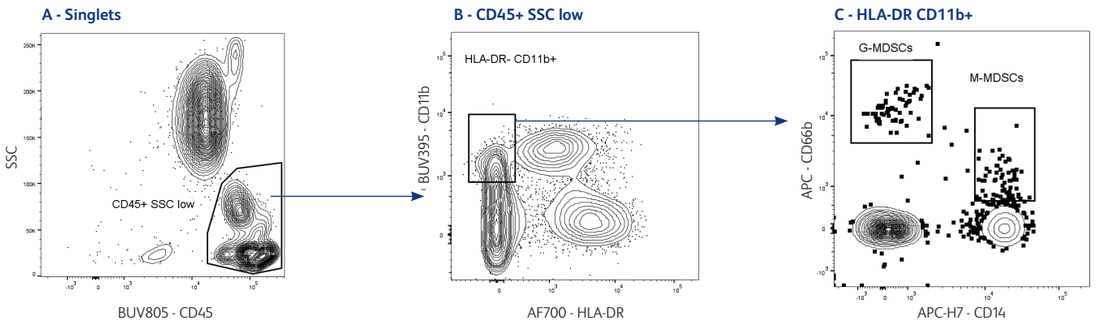


Figure 11 - Human whole blood was stained with the Myeloid Backbone Panel Block (Table 10) and the Myeloid-derived Suppressor Cell Panel Block (Table 13) and acquired on a 5-laser BD FACSymphony™ A3 Cell Analyzer. Performance was also tested on the BD FACSymphony™ A5 and BD FACSymphony™ A5 SE Cell Analyzers. Clear resolution of the cell subsets was observed irrespective of the instrument utilized (not shown) Initial gating was done from singlets.

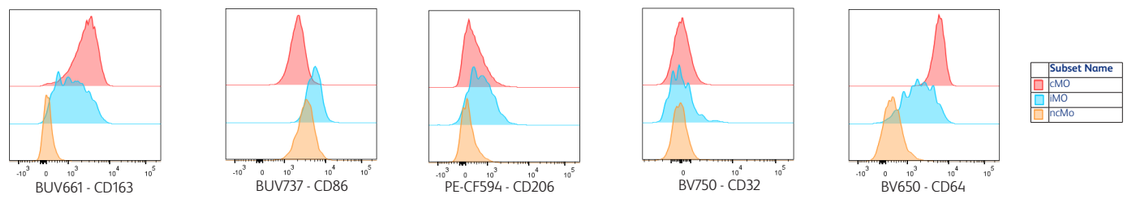
Myeloid Activation Panel Block

Myeloid cells are constantly monitoring the body for signs of pathogens and tissue damage and are often considered the body's first line of defense. Myeloid cells will be activated by several different types of receptors, depending on the nature of the signal and the cell type. This panel consists of markers with diverse functions that have been implicated in overall activation of circulating myeloid cells or as more anti-inflammatory.[16,17,18,19,20] Dysregulation of these markers has even been implicated in diseases such as cancer, sepsis and autoimmune disease.[21,22,23]

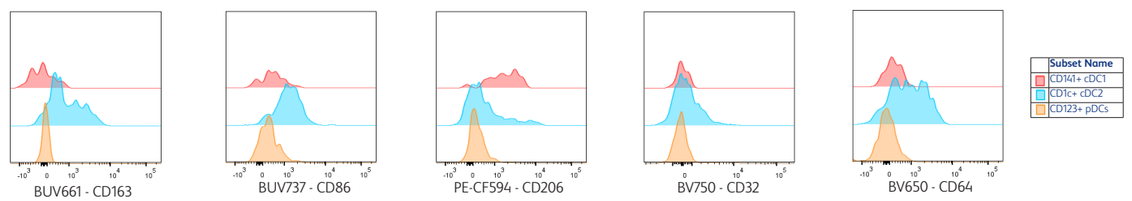
Laser Line	Fluorochrome	Marker	Clone	Volume Per Test	Catalog Number
Ultra-Violet 355 nm	BUV661	CD163	GHI/61	1 µl	741645
	BUV737	CD86	BU63	1 µl	748376
Violet 405 nm	BV650	CD64	10.1	1 µl	740580
	BV750	CD32	3D3	1 µl	747110
Yellow-Green 561 nm	PE-CF594	CD206	19.2	5 µl	564063

Table 14. Myeloid Activation Panel Block. Recommended volume per test was determined using 100ul of human whole blood.

Marker expression on different subpopulations of monocytes



Marker expression on different subpopulations of dendritic cells



Protocols

tips&tricks

Sign up for a free BD Research Cloud account to explore these panel blocks, and easily add drop-in reagents for your experiment.

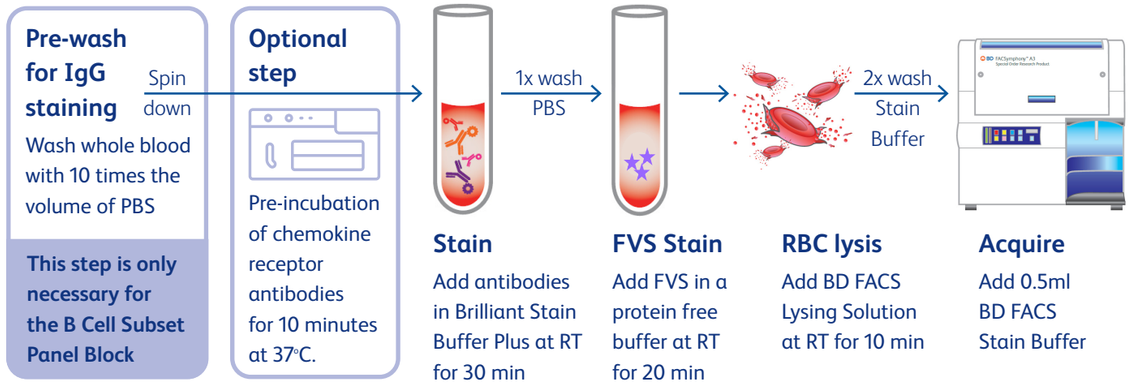
Use the BD Horizon™ Brilliant Stain Buffer to ensure optimal performance.

BD Human Fc Block™ Reagent should be added when nonspecific Fc binding is of concern, such as when using antibodies with IgG2a isotype.

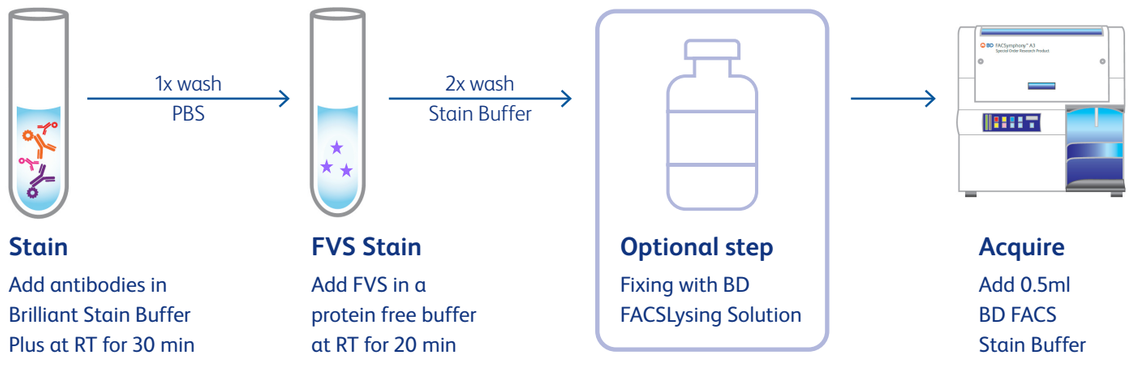
Antibody staining

Different workflows can be used to stain cells, depending on the type of sample used.

Staining Whole Blood



Staining PBMC or enriched cells



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