

BD Flow Cytometry Reagents

Multicolor Panels Designed for Optimal Resolution with the BD FACSVerserTM Flow Cytometer

Proper multicolor panel design takes into account fluorochrome brightness, antigen density, co-expression, and spillover in the context of a given instrument configuration. The following experiments were designed to maximize resolution of a variety of cell types on a BD FACSVerserTM flow cytometer.

BD FACSVerser Configuration

Laser	Fluorochrome	Filter
Violet (405 nm)	BD Horizon TM BV421	448/45
	BD Horizon TM BV510 BD Horizon TM Fixable Viability Stain 510	528/45
	BD Horizon TM BB515	
Blue (488 nm)	FITC	527/32
	Alexa Fluor [®] 488	
	PE	586/42
	PerCP-Cy TM 5.5	700/54
Red (640 nm)	PE-Cy TM 7	783/56
	APC	
	Alexa Fluor [®] 647	660/10
	APC-H7	
	APC-Cy7	783/56

Similar configurations exist on the following instruments: BD LSRFortessaTM, BD FACSAriaTM Fusion, BD FACSAriaTM III, BD FACSCantoTM II, BD InfluxTM.



B-Cell Panel

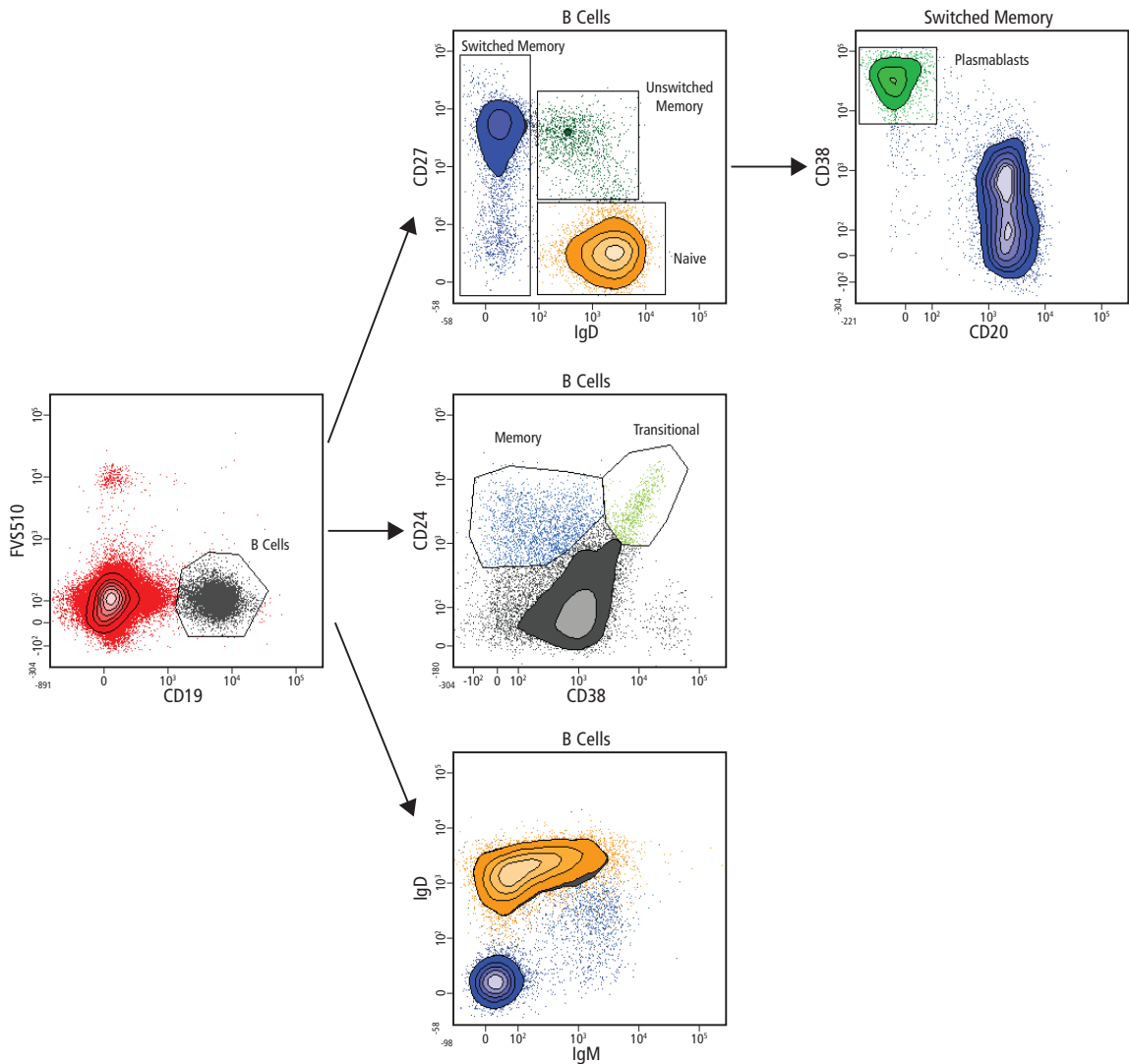
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD19	SJ25C1	PE-Cy7	B cells	557835
IgM	G20-127	PE	B-cell subset	555783
IgD	IA6-2	FITC	B-cell subset	555778
CD24	ML5	Alexa Fluor® 647	Memory/naïve subset	561644
CD27	M-T271	BD Horizon BV421	Memory subset	562513
CD20	2H7	APC-H7	Plasmablast subset	560734
CD38	HIT2	PerCP-Cy5.5	Plasmablast subset	551400
Viability	N/A	BD Horizon Fixable Viability Stain 510	Cellular viability	564406

Protocol

PBMCs were prepared by using Ficoll-Paque™ Plus according to the manufacturer’s directions and then frozen. Thawed PBMCs were washed and incubated with antibodies in the presence of BD Horizon™ Brilliant Stain Buffer at room temperature, protected from light, for 20 minutes and washed prior to acquisition.

Data

Analysis of Human B-Cell Subsets



T-Cell Panel

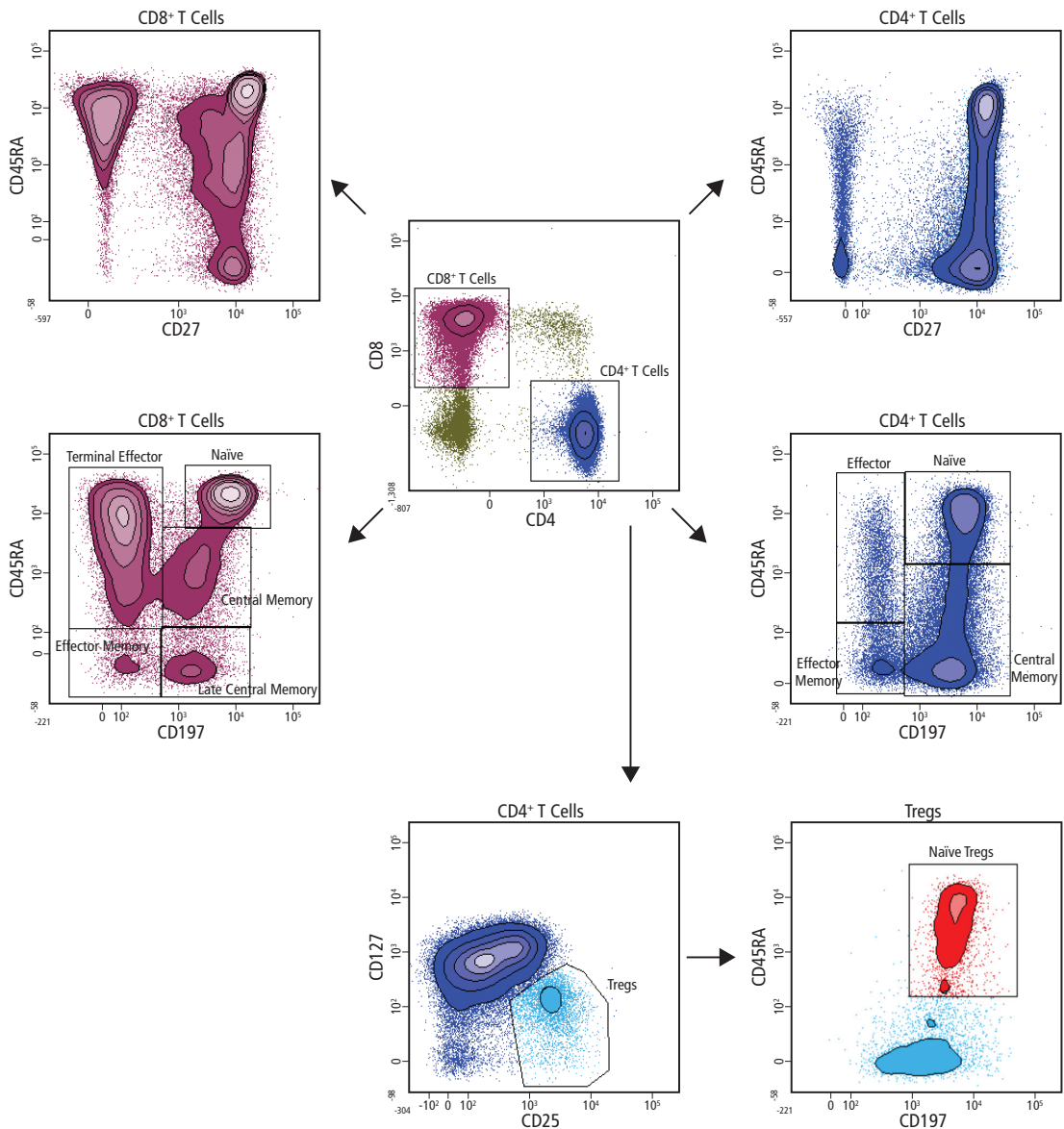
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	BD Horizon BV510	T cell	563109
CD4	RPA-T4	APC-H7	Helper T-cell subset	560158
CD8	RPA-T8	PerCP-Cy5.5	Cytotoxic T-cell subset	560662
CD127	HIL-7R-M21	Alexa Fluor® 647	Treg identification	558598
CD25	M-A251	PE-Cy7	Treg identification	557741
CD27	M-T271	PE	Antigen experience	555441
CD45RA	HI100	BD Horizon BB515	Antigen experience	564552
CD197/CCR7	150503	BD Horizon BV421	Antigen experience	562555

Protocol

Peripheral blood was stained with antibodies in the presence of BD Horizon Brilliant Stain Buffer at room temperature, protected from light, for 20 minutes. Samples were subsequently lysed with BD FACST[™] Lysing Solution and washed prior to acquisition.

Data

Analysis of Human T-Cell Populations



Naïve, Effector, and Memory T-Cell Panel

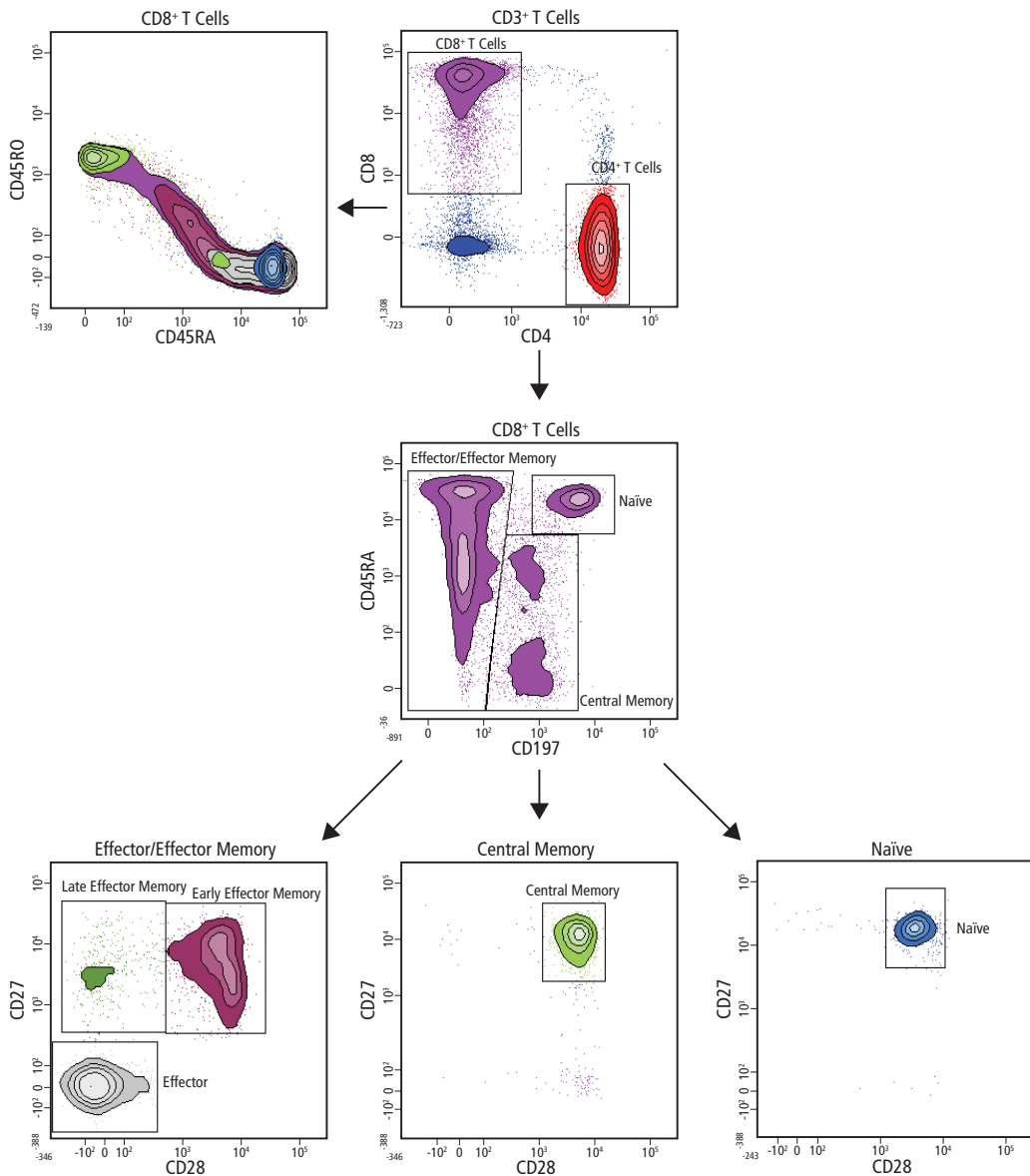
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	BD Horizon BV510	T cells	563109
CD4	RPA-T4	PerCP-Cy5.5	CD4 ⁺ subset	560650
CD8	SK1	APC-H7	CD8 ⁺ subset	560179
CD27	M-T271	BD Horizon BV421	Antigen experience	562513
CD28	CD28.2	APC	Antigen experience	559770
CD45RA	HI100	BD Horizon BB515	Antigen experience	564552
CD45RO	UCHL1	PE-Cy7	Antigen experience	560608
CD197/CCR7	150503	PE	Antigen experience	560765

Protocol

Peripheral blood was stained with antibodies in the presence of BD Horizon Brilliant Stain Buffer at room temperature, protected from light, for 20 minutes. Samples were subsequently lysed with BD FACS Lysing Solution and washed prior to acquisition.

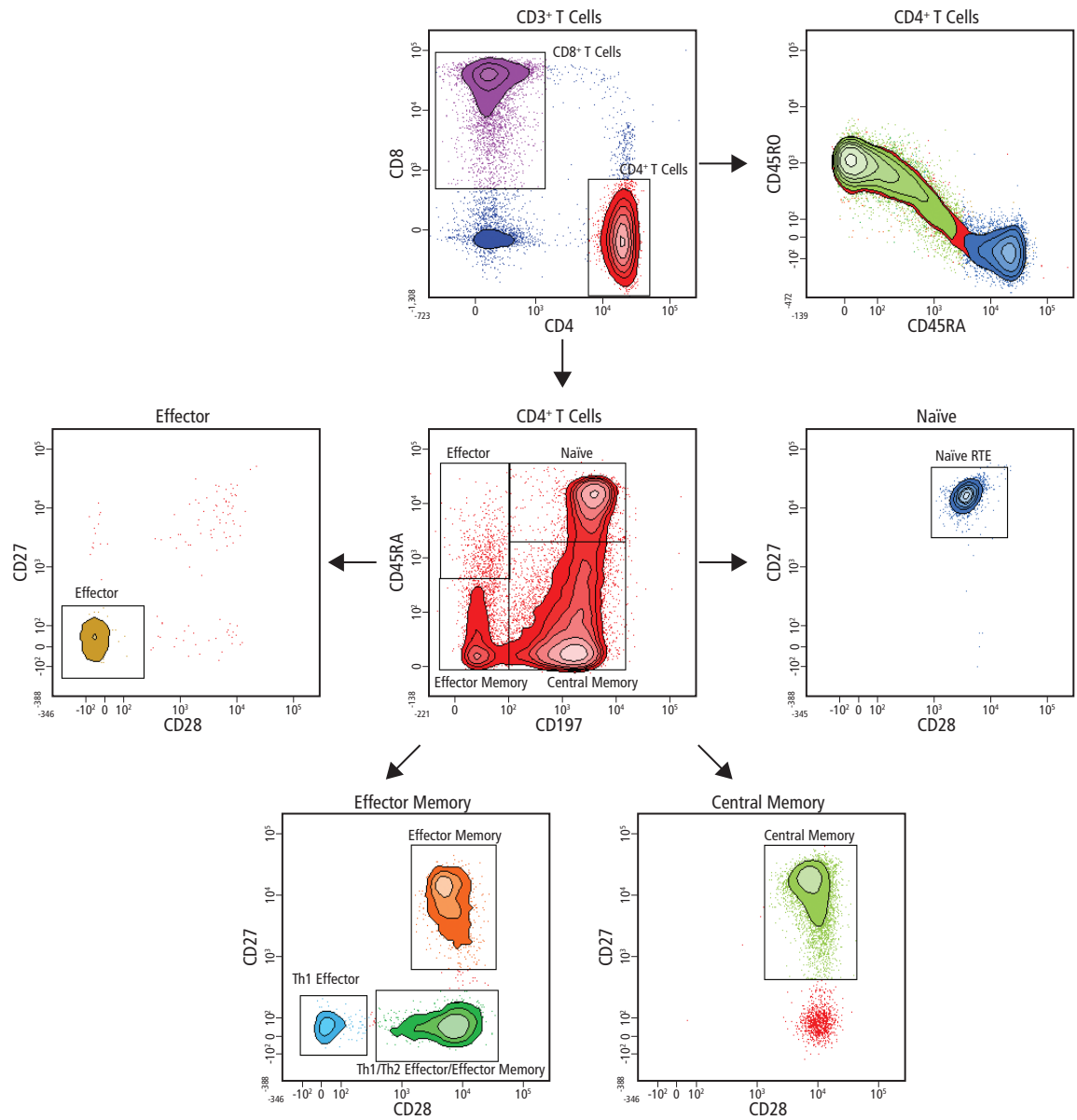
Data

Analysis of Human Naïve, Effector, and Memory T-Cell Populations (CD8⁺)



Data

Analysis of Human Naive, Effector, and Memory T-Cell Populations (CD4⁺)



Regulatory T-Cell (Treg) Panel

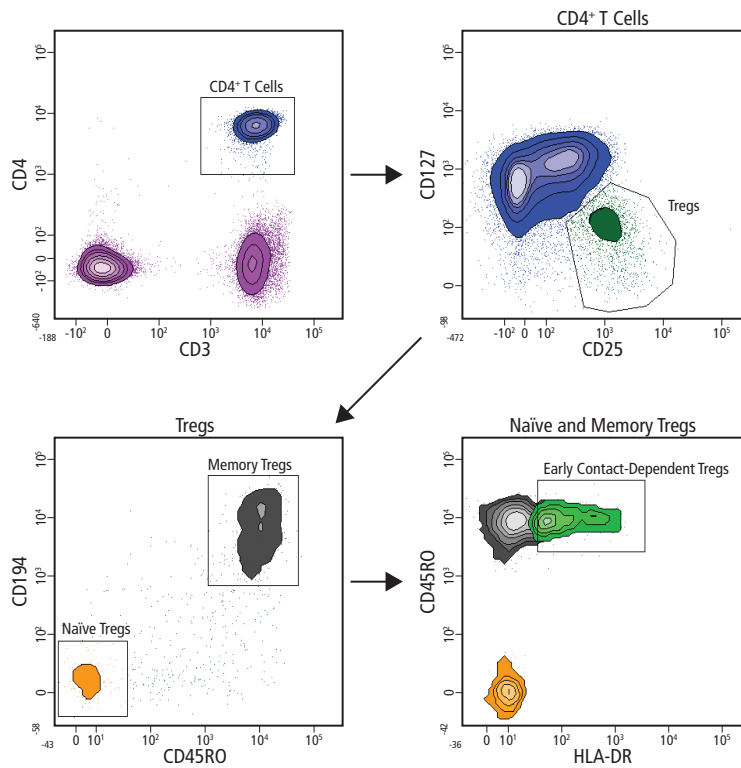
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	PerCP-Cy5.5	T cell	560835
CD4	RPA-T4	APC-H7	Helper T-cell subset	560158
CD25	M-A251	PE	Treg identification	555432
CD127	HIL-7R-M21	Alexa Fluor® 647	Treg identification	558598
HLA-DR	G46-6	BD Horizon BV510	Treg subset	563083
CD194/CCR4	1G1	BD Horizon BV421	Antigen experience	562579
CD45RO	UCHL1	BD Horizon BB515	Antigen experience	564529

Protocol

Peripheral blood was stained with antibodies in the presence of BD Horizon Brilliant Stain Buffer at room temperature, protected from light, for 20 minutes. Samples were subsequently lysed with BD FACS Lysing Solution and washed prior to acquisition.

Data

Analysis of Human Treg Populations



Dendritic Cell Panel

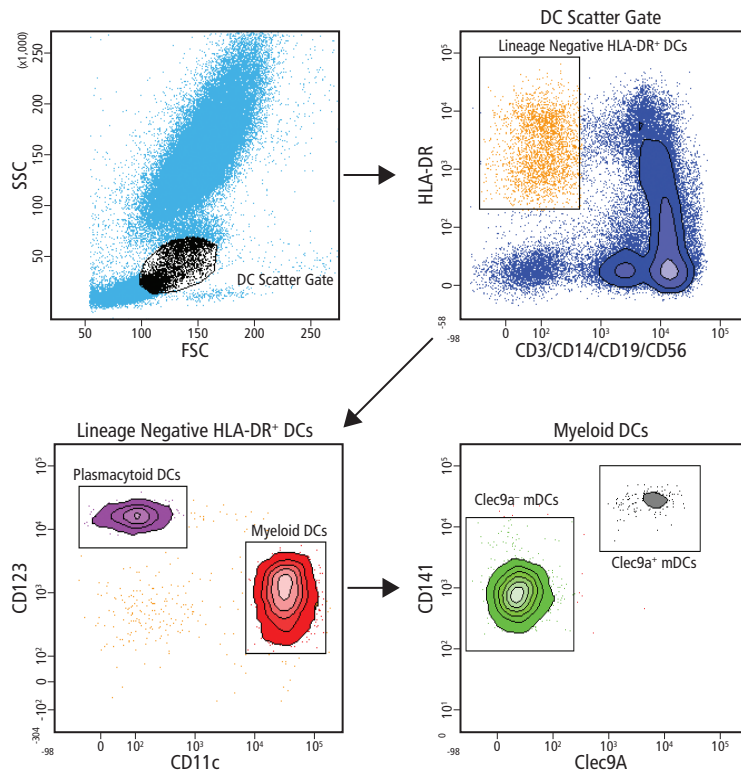
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	PerCP-Cy5.5	Exclusion (T cell)	560835
CD14	MφP9	PerCP-Cy5.5	Exclusion (monocyte)	562692
CD19	HIB19	PerCP-Cy5.5	Exclusion (B cell)	561295
CD56	B159	PerCP-Cy5.5	Exclusion (granulocyte)	560842
HLA-DR	G46.6	BD Horizon BV510	Dendritic cells (DCs)	563083
CD11c	B-ly6	BD Horizon BV421	Myeloid DCs (mDCs)	562561
Clec9a	3A4/Clec9A	PE	mDC subset	563488
CD141	1A4	APC	mDC subset	564123
CD123	7G3	PE-Cy7	Plasmacytoid DCs (pDCs)	560826

Protocol

Peripheral blood was stained with antibodies in the presence of BD Horizon Brilliant Stain Buffer at room temperature, protected from light, for 20 minutes. Samples were subsequently lysed with BD FACS Lysing Solution and washed prior to acquisition.

Data

Analysis of Human Dendritic Cell Populations



Myeloid Cell Panel

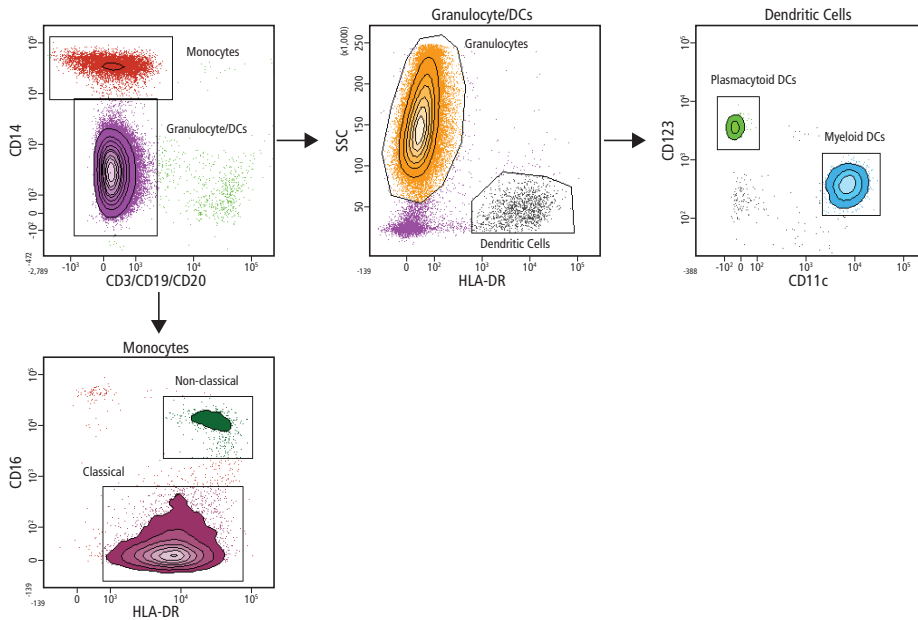
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	SK7	APC-H7	Exclusion (T cell)	560176
CD19	SI25C1	APC-H7	Exclusion (B cell)	560177
CD20	2H7	APC-H7	Exclusion (B cell)	560853
HLA-DR	G46-6	PE	DC, NK, monocyte subsets	555812
CD16	3G8	BD Horizon BV421	NK cell/monocyte subset	562874
CD56	NCAM 16.2	BD Horizon BB515	NK cells	564488
CD11c	B-Ly6	APC	DC subset	559877
CD123	7G3	BD Horizon BV510	pDC subset	563072
CD14	MφP9	PE-Cy7	Monocytes	562698

Protocol

Peripheral blood was stained with antibodies in the presence of BD Horizon Brilliant Stain Buffer at room temperature, protected from light, for 20 minutes. Samples were subsequently lysed with BD FACS Lysing Solution and washed prior to acquisition.

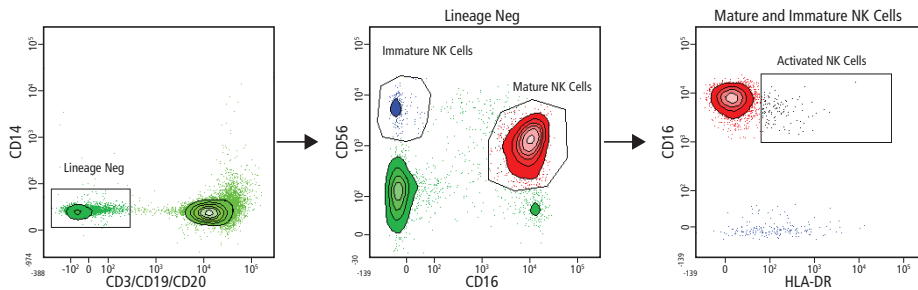
Data

Analysis of Human Myeloid Cells (dendritic cells (DCs) and monocytes)



Data

Analysis of Human Natural Killer (NK) Cells



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 23-17125-00

