

BD Flow Cytometry Reagents

Multicolor Panels Designed for Optimal Resolution

with the BD LSRFortessa™ X-20 Cell Analyzer

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 LSRFortessa™ X-20 cell analyzer.

BD LSRFortessa™ X-20 Configuration

Laser	Fluorochrome	Filter
Ultraviolet (355 nm)	BD Horizon™ BUV395	379/28
	BD Horizon™ BUV496	515/30
	BD Horizon™ BUV661	670/25
	BD Horizon™ BUV737	740/35
Violet (405 nm)	BD Horizon™ BV421	450/50
	BD Horizon™ BV510	525/50
	BD Horizon™ BV605	610/20
	BD Horizon™ BV650	670/30
	BD Horizon™ BV711	710/50
Blue (488 nm)	BD Horizon™ BB515	530/30
	FITC	
	Alexa Fluor® 488	
	BD Horizon™ Fixable Viability Stain 520	
Yellow-Green (561 nm)	PerCP-Cy™5.5	670/30
	PE	586/15
	BD Horizon™ PE-CF594	610/20
Red (640 nm)	PE-Cy™7	780/60
	APC	670/30
	Alexa Fluor® 647	
	BD Horizon™ APC-R700	730/45
Alexa Fluor® 700		
	APC-H7	780/60
	APC-Cy7	

Similar configurations exist on the following instruments: BD LSRFortessa™ cell analyzer, BD FACSAria™ Fusion cell sorter, and BD FACSAria™ III cell sorter.



B-Cell Panel

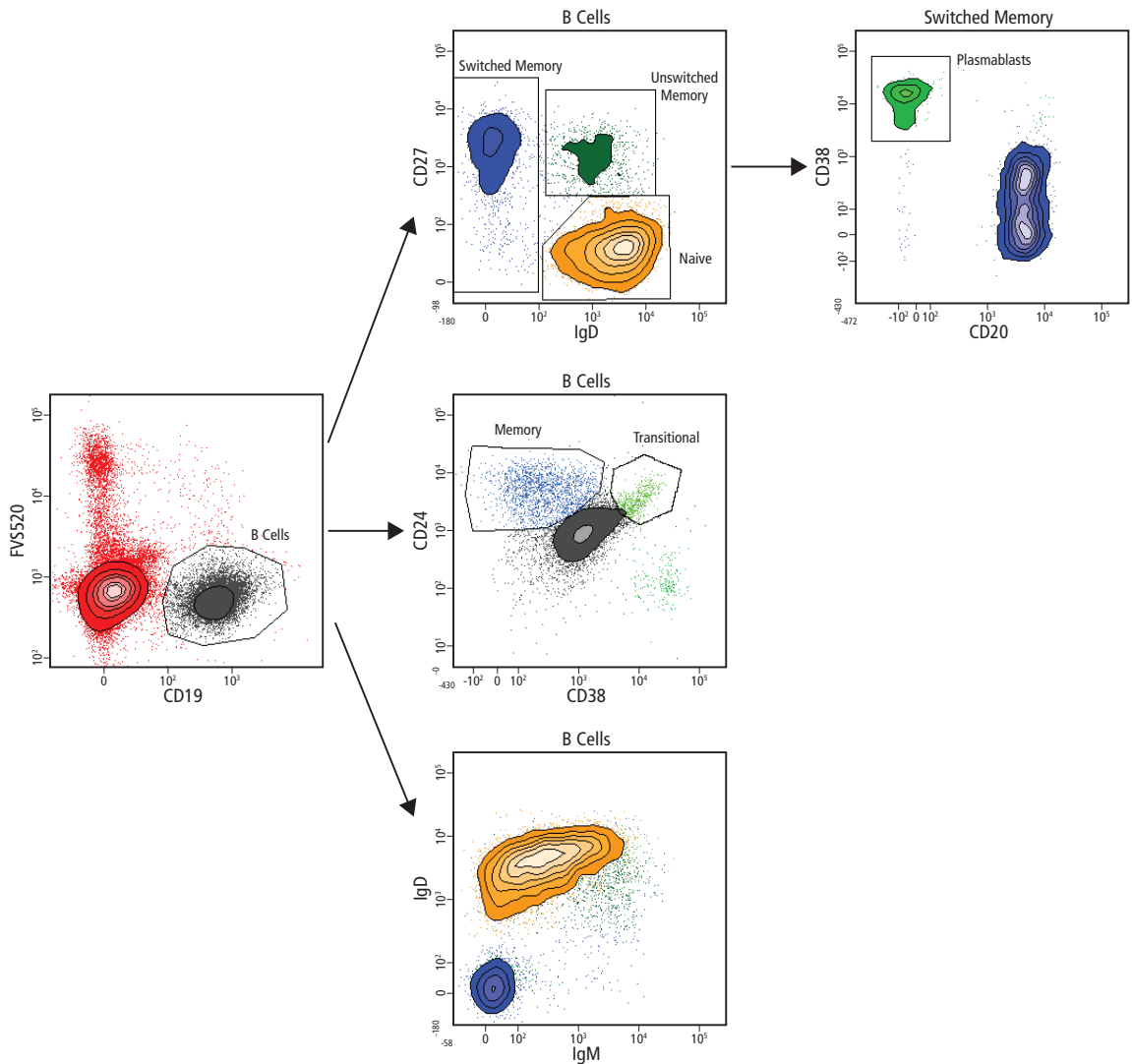
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD19	HIB19	BD Horizon™ BUV496	B cells	561295
IgM	G20-127	PE	B-cell subset	555783
IgD	IA6-2	BD Horizon™ BUV395	B-cell subset	563813
CD24	ML5	BD Horizon™ BV510	Memory/naïve subset	563035
CD27	M-T271	BD Horizon™ BV421	Memory subset	562513
CD20	2H7	APC-H7	Plasmablast subset	560734
CD38	HIT2	PerCP-Cy™5.5	Plasmablast subset	551400
Viability	n/a	BD Horizon™ Fixable Viability Stain 520	Cellular viability	564407

Protocol

PBMCs were prepared by using Ficoll-Paque™ Plus according to the manufacturer’s directions 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

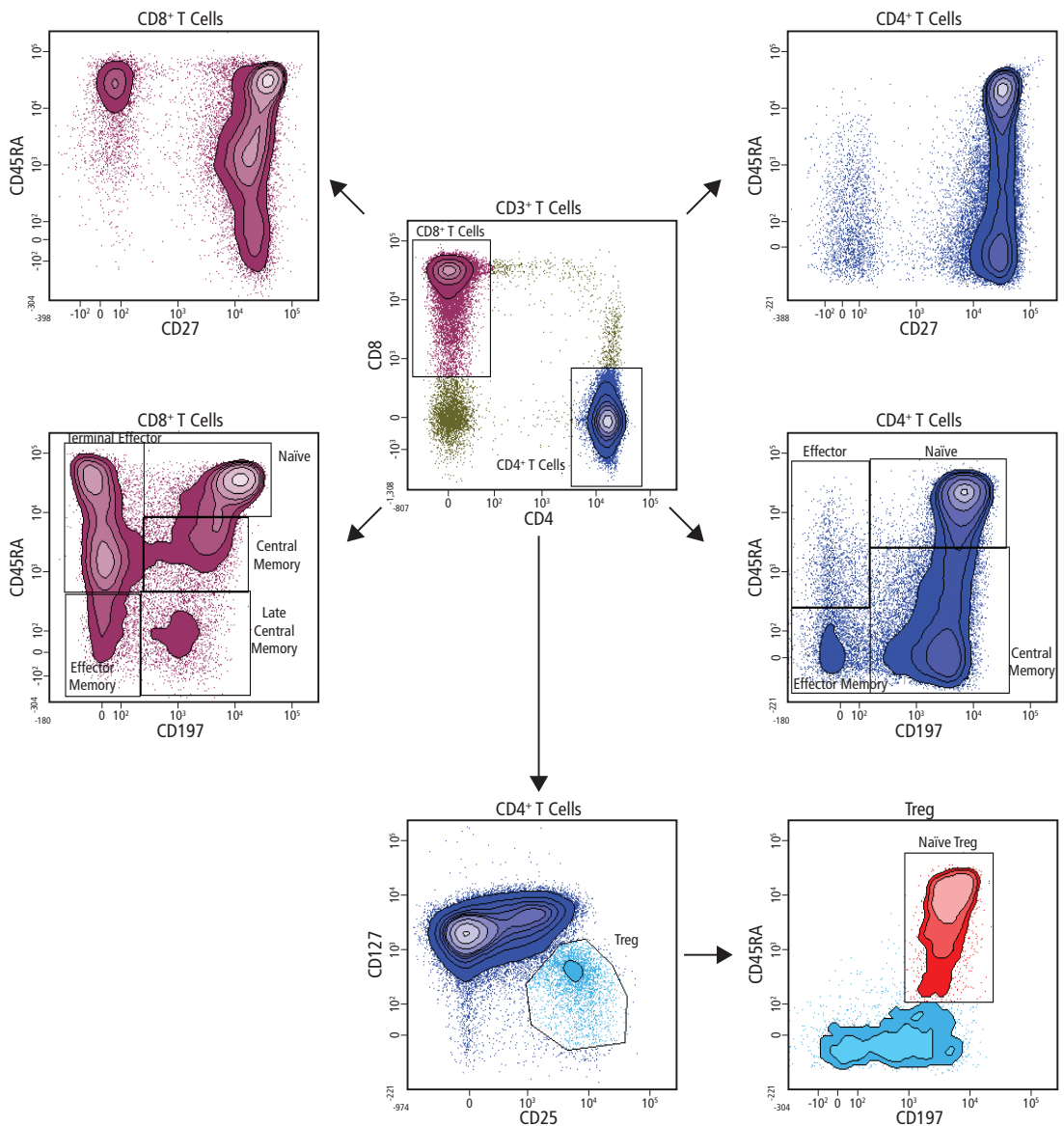
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	BD Horizon™ BB515	T cells	564465
CD4	SK3	BD Horizon™ BUV395	Helper T-cell subset	563550
CD8	SK1	APC-H7	Cytotoxic T-cell subset	560179
CD127	HIL-7R-M21	BD Horizon™ BV786	Treg identification	563324
CD25	M-A251	PE	Treg identification	555432
CD27	M-T271	BD Horizon™ BV421	Antigen experience	562513
CD45RA	HI100	APC	Antigen experience	550855
CD197/CCR7	150503	BD Horizon™ PE-CF594	Antigen experience	562381

Protocol

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

Data

Analysis of Human T-Cell Populations



Naïve, Effector, and Memory T-Cell Panel

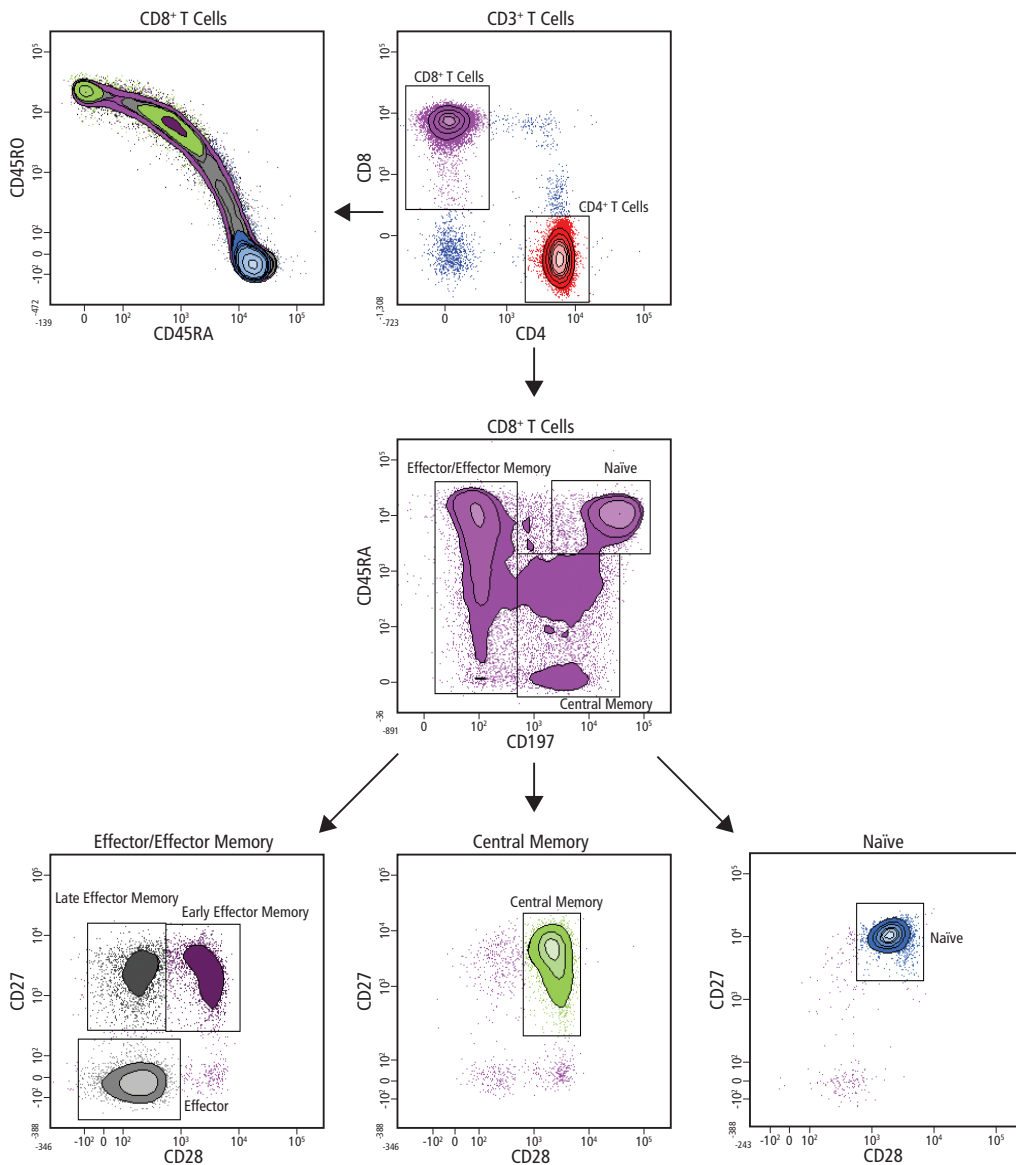
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	PerCP-Cy5.5	T cells	560835
CD4	SK3	BD Horizon™ BUV496	CD4+ subset	564651
CD8	SK1	APC-H7	CD8+ subset	560179
CD27	M-T271	BD Horizon™ BV421	Antigen experience	562513
CD28	CD28.2	APC	Antigen experience	559770
CD45RA	HI100	FITC	Antigen experience	555488
CD45RO	UCHL1	BD Horizon™ BUV395	Antigen experience	564291
CD197/CCR7	150503	BD Horizon™ PE-CF594	Antigen experience	562381

Protocol

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

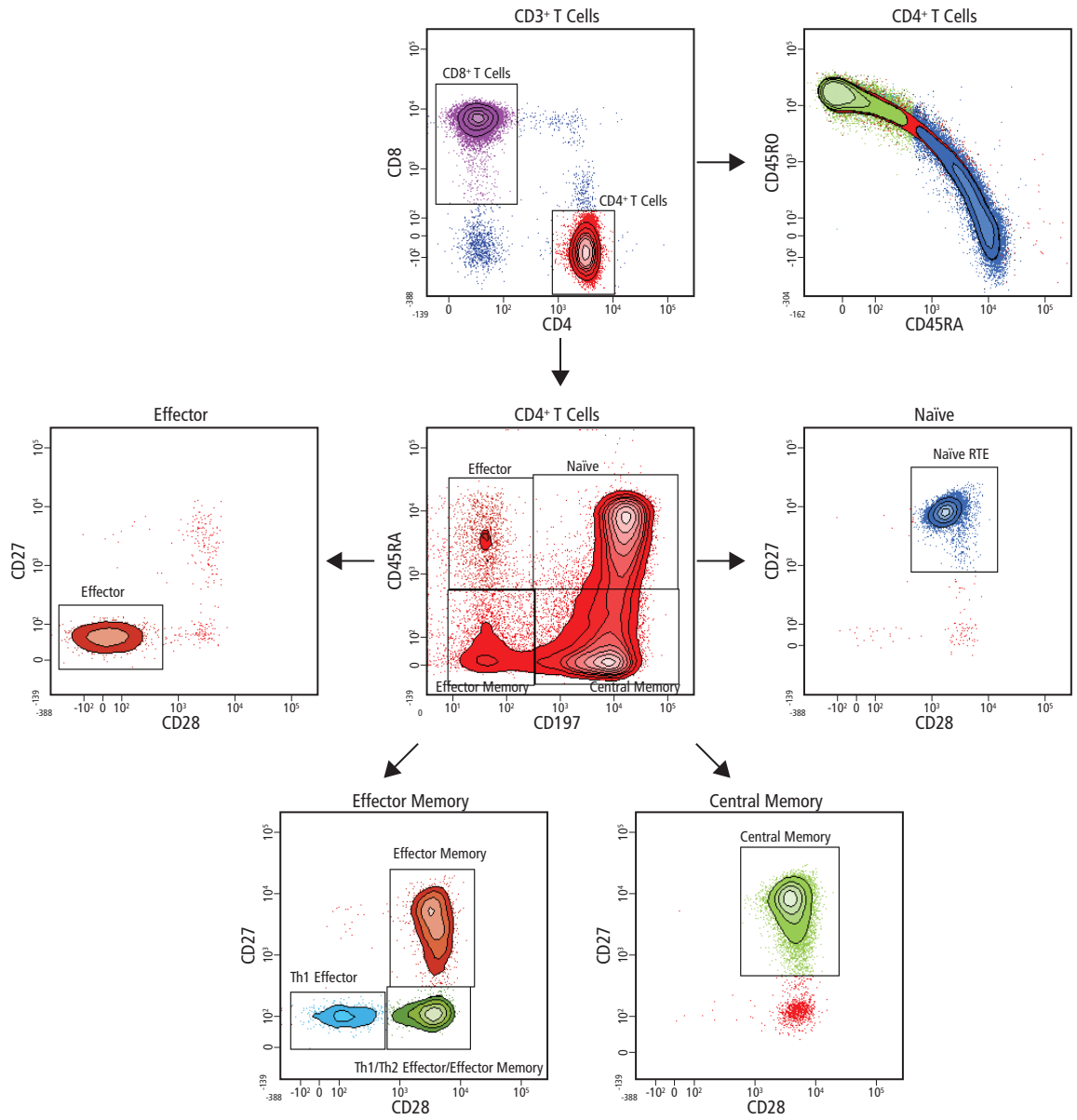
Data

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

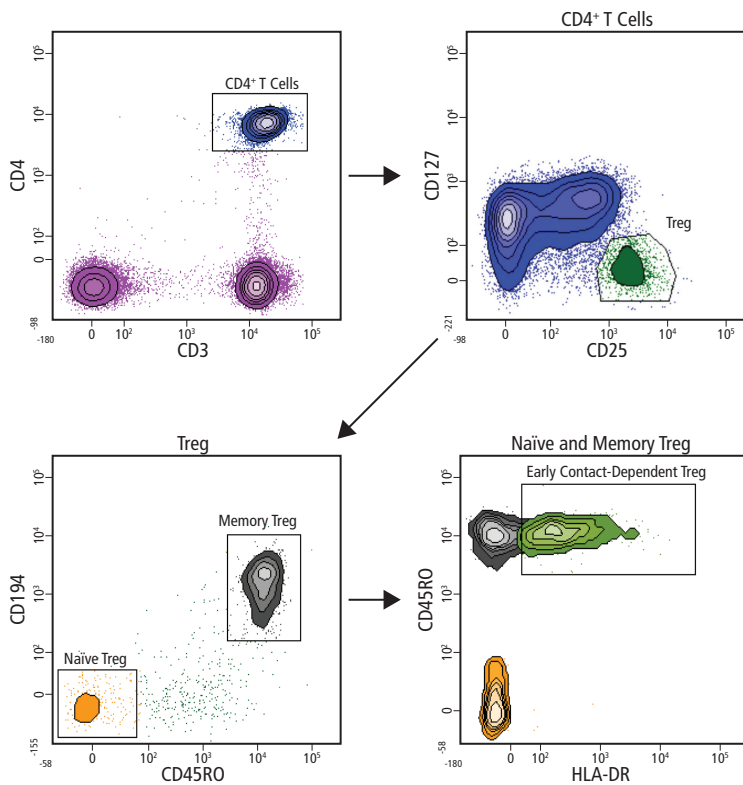
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	BD Horizon™ BV711	T cells	563725
CD4	SK3	BD Horizon™ BUV395	Helper T-cell subset	563550
CD25	M-A251	BD Horizon™ PE-CF594	Treg identification	562403
CD127	HIL-7R-M21	Alexa Fluor® 647	Treg identification	558598
HLA-DR	G46-6	PE-Cy7	Treg subset	560651
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 and protected from light for 20 minutes. Samples were subsequently lysed with BD FACST™ Lysing Solution and washed prior to acquisition.

Data

Analysis of Human Treg Populations



Dendritic Cell Panel

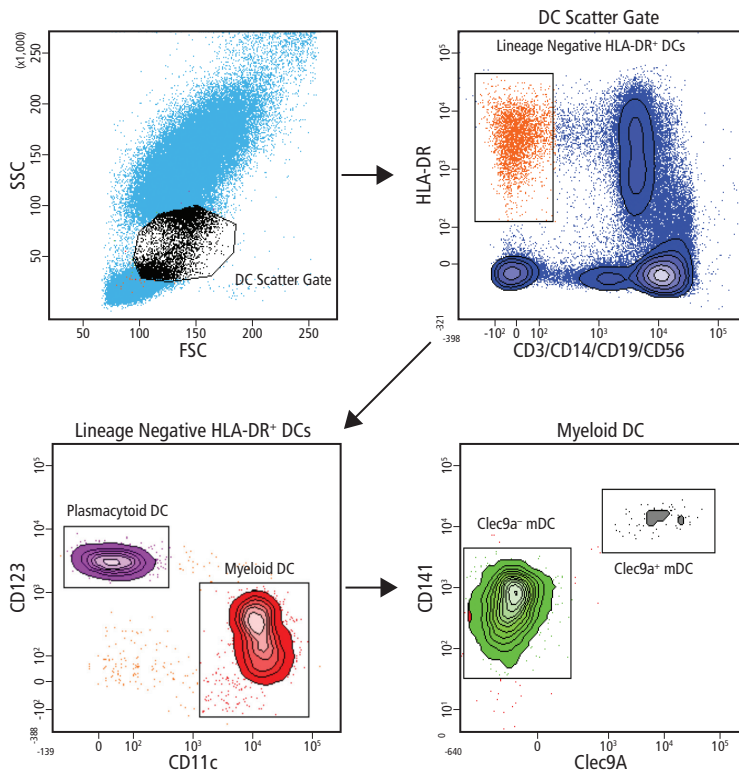
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	SK7	BD Horizon™ BV786	Exclusion (T cell)	563799
CD14	M5E2	BD Horizon™ BV786	Exclusion (monocyte)	563698
CD19	SJ25C1	BD Horizon™ BV786	Exclusion (B cell)	563325
CD56	NCAM16.2	BD Horizon™ BV786	Exclusion (granulocyte)	564058
HLA-DR	G46.6	BD Horizon™ BV421	Dendritic cells (DC)	562804
CD11c	B-ly6	BD Horizon™ BB515	Myeloid DC (mDC)	564490
Clec9a	3A4/Clec9A	PE	mDC Subset	563488
CD141	1A4	APC	mDC Subset	564123
CD123	7G3	BD Horizon™ BUV395	Plasmacytoid DC (pDC)	564195

Protocol

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

Data

Analysis of Human Dendritic Cell Populations



Myeloid Cell Panel

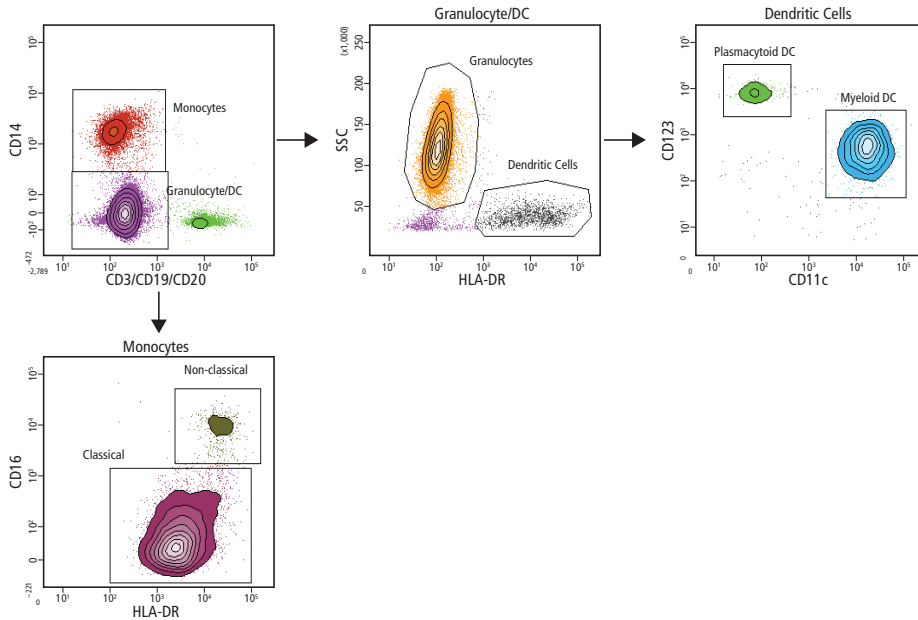
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	BD Horizon™ BV510	Exclusion (T cell)	563109
CD19	SJ25C1	BD Horizon™ BV510	Exclusion (B cell)	562947
CD20	2H7	BD Horizon™ BV510	Exclusion (B cell)	563067
HLA-DR	G46-6	FITC	DC, NK, monocyte subsets	555811
CD16	3G8	BD Horizon™ BUV737	NK cell/monocyte subset	564434
CD56	NCAM 16.2	BD Horizon™ BV421	NK cells	562751
CD11c	B-Ly6	BD Horizon™ PE-CF594	DC subset	562393
CD123	7G3	BD Horizon™ BUV395	pDC subset	564195
CD14	M5E2	APC	Monocytes	555399

Protocol

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

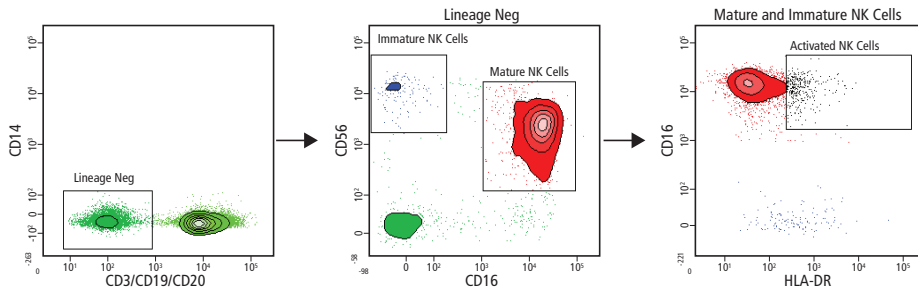
Data

Analysis of Myeloid Cells (Dendritic Cells (DC) and Monocytes)



Data

Analysis of Human Natural Killer (NK) Cells



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