

# 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**

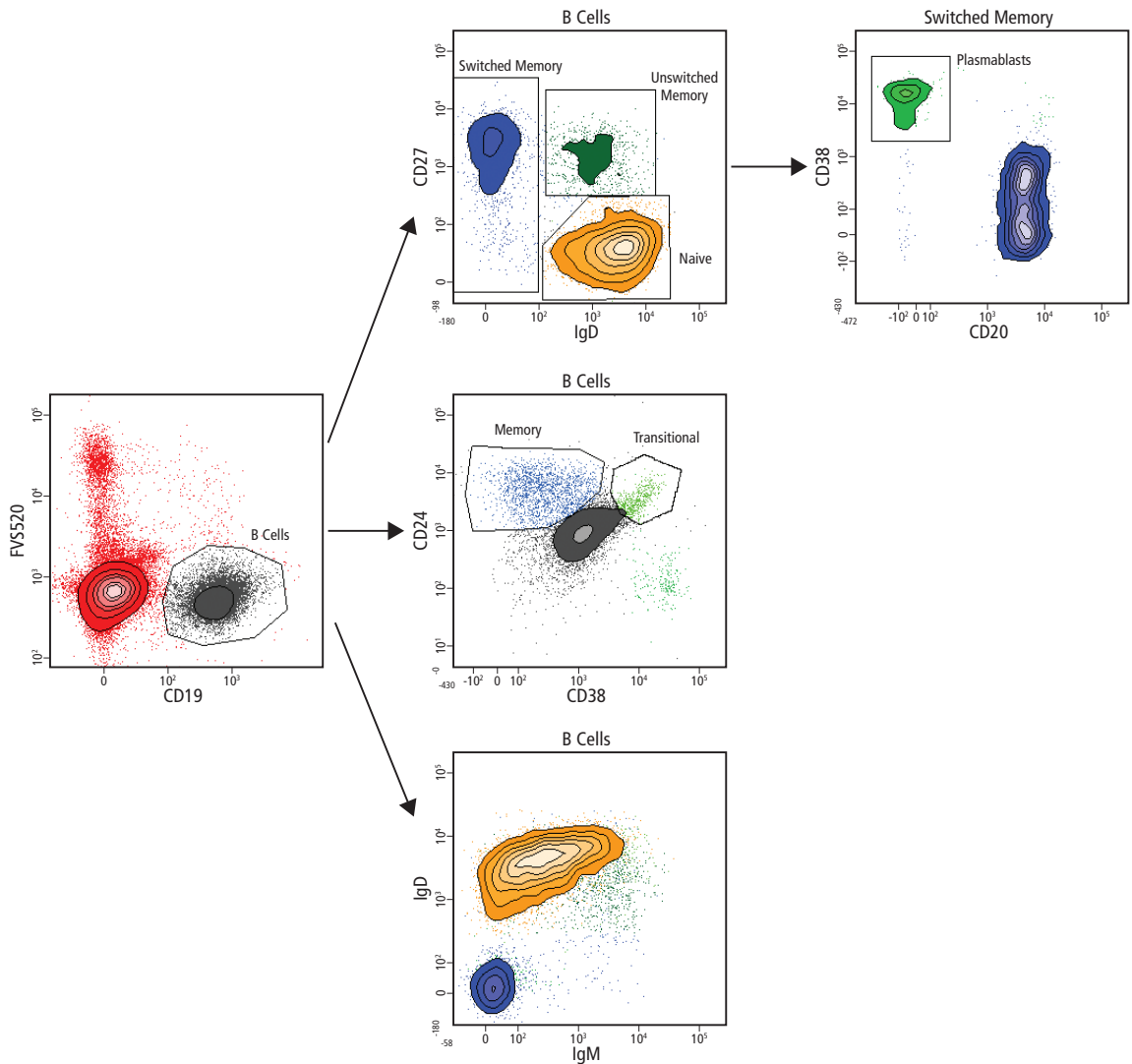
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD19	HIB19	BD Horizon™ BUV496	B cells	<b>561295</b>
IgM	G20-127	PE	B-cell subset	<b>555783</b>
IgD	IA6-2	BD Horizon™ BUV395	B-cell subset	<b>563813</b>
CD24	ML5	BD Horizon™ BV510	Memory/naïve subset	<b>563035</b>
CD27	M-T271	BD Horizon™ BV421	Memory subset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

**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**

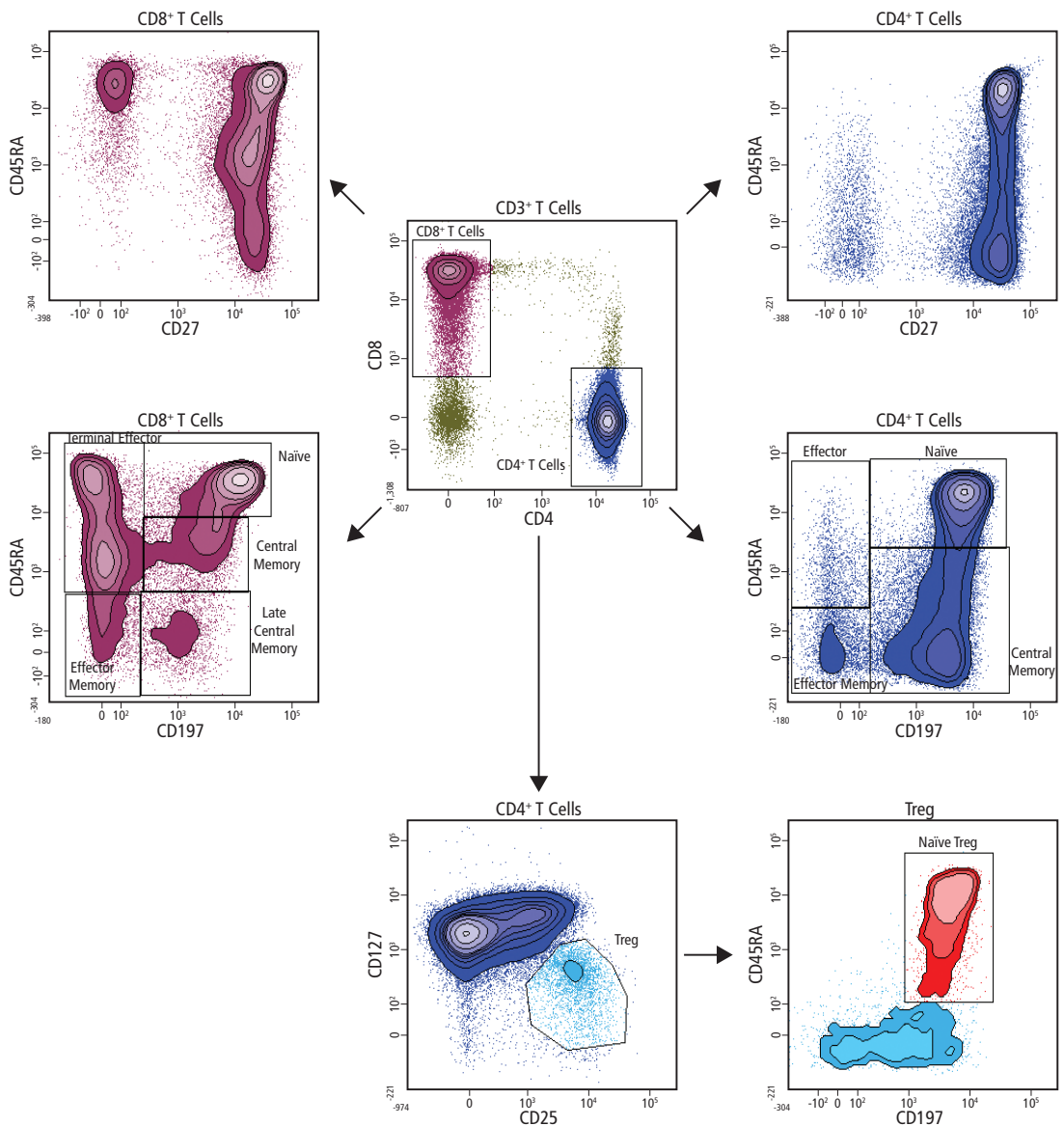
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	BD Horizon™ BB515	T cells	<b>564465</b>
CD4	SK3	BD Horizon™ BUV395	Helper T-cell subset	<b>563550</b>
CD8	SK1	APC-H7	Cytotoxic T-cell subset	<b>560179</b>
CD127	HIL-7R-M21	BD Horizon™ BV786	Treg identification	<b>563324</b>
CD25	M-A251	PE	Treg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

**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**

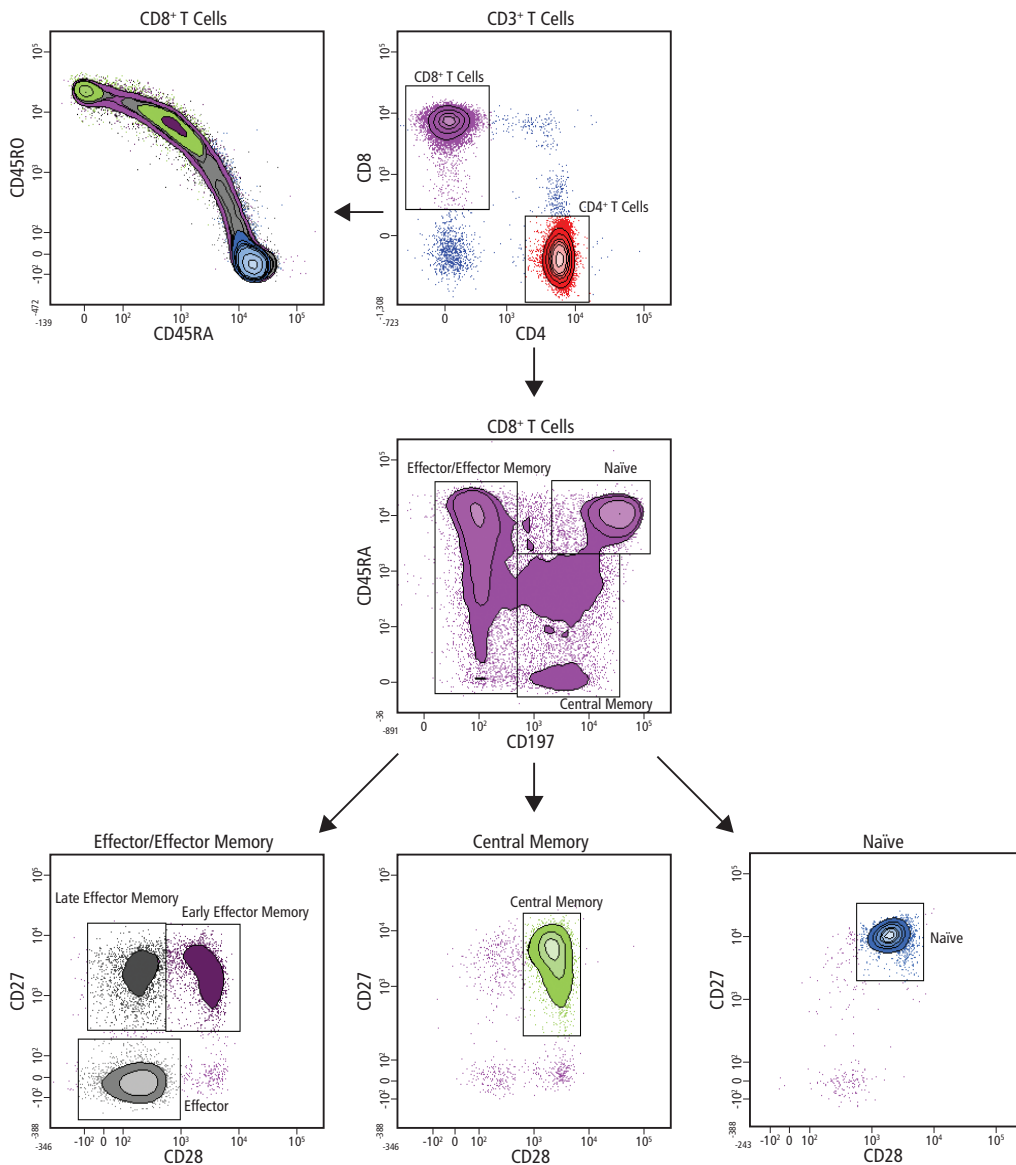
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	PerCP-Cy5.5	T cells	<b>560835</b>
CD4	SK3	BD Horizon™ BUV496	CD4+ subset	<b>564651</b>
CD8	SK1	APC-H7	CD8+ subset	<b>560179</b>
CD27	M-T271	BD Horizon™ BV421	Antigen experience	<b>562513</b>
CD28	CD28.2	APC	Antigen experience	<b>559770</b>
CD45RA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

**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.

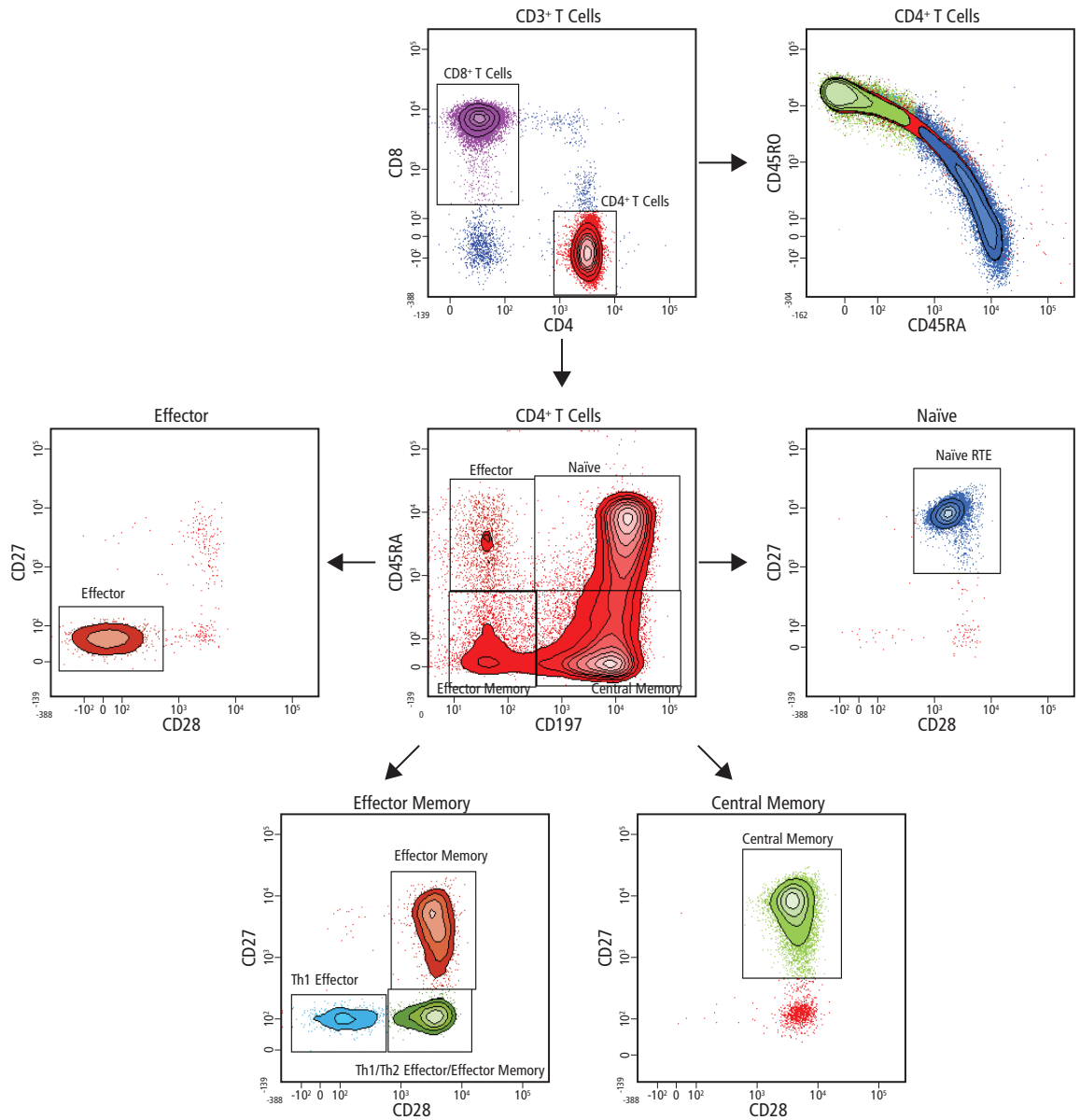
**Data**

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



Data

Analysis of Human Naive, Effector, and Memory T Cell Populations (CD4<sup>+</sup>)



**Regulatory T-Cell (Treg) Panel**

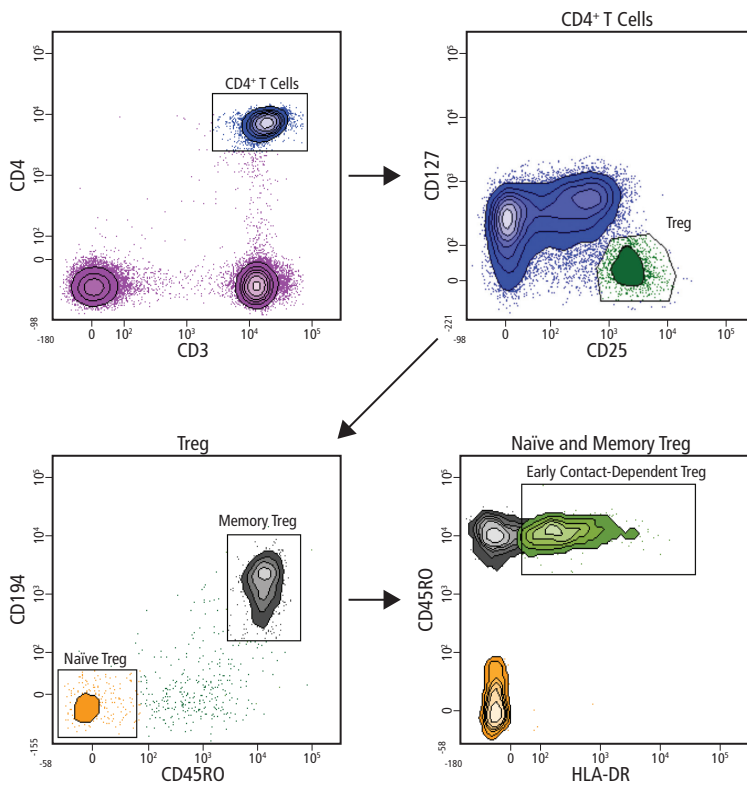
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	BD Horizon™ BV711	T cells	<b>563725</b>
CD4	SK3	BD Horizon™ BUV395	Helper T-cell subset	<b>563550</b>
CD25	M-A251	BD Horizon™ PE-CF594	Treg identification	<b>562403</b>
CD127	HIL-7R-M21	Alexa Fluor® 647	Treg identification	<b>558598</b>
HLA-DR	G46-6	PE-Cy7	Treg subset	<b>560651</b>
CD194/CCR4	1G1	BD Horizon™ BV421	Antigen experience	<b>562579</b>
CD45RO	UCHL1	BD Horizon™ BB515	Antigen experience	<b>564529</b>

**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**

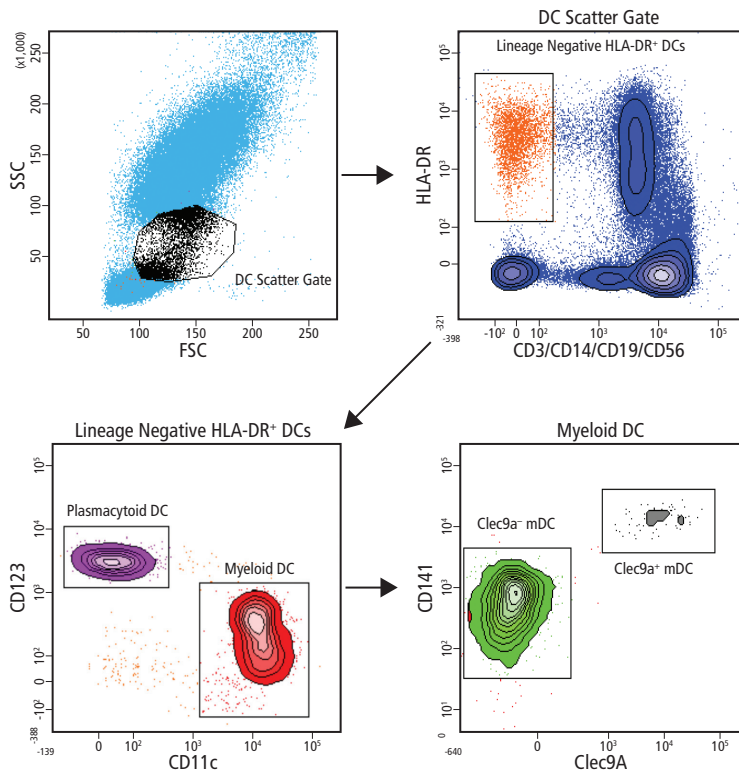
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	SK7	BD Horizon™ BV786	Exclusion (T cell)	<b>563799</b>
CD14	M5E2	BD Horizon™ BV786	Exclusion (monocyte)	<b>563698</b>
CD19	SJ25C1	BD Horizon™ BV786	Exclusion (B cell)	<b>563325</b>
CD56	NCAM16.2	BD Horizon™ BV786	Exclusion (granulocyte)	<b>564058</b>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

**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**

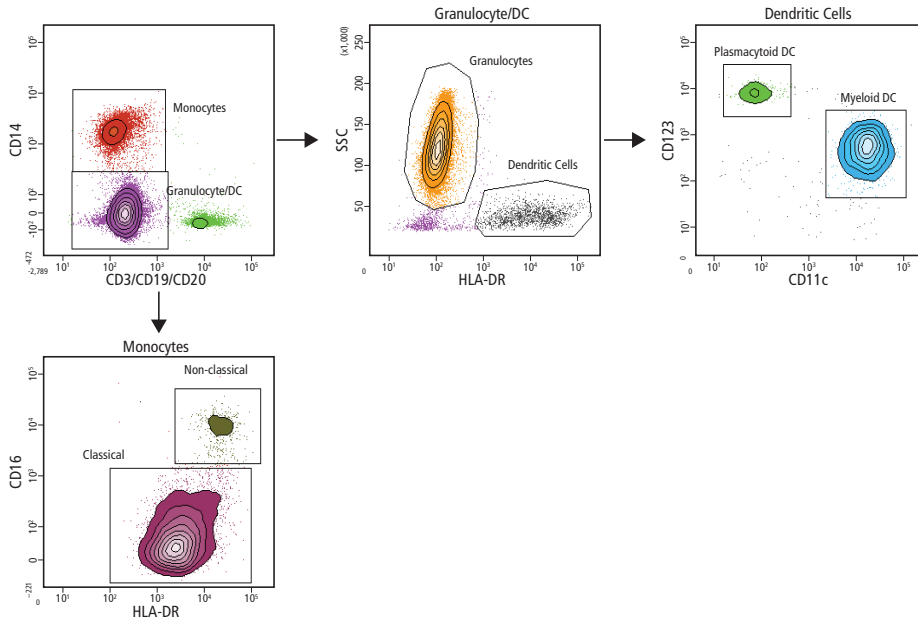
Specificity	Clone	Fluorochrome	Purpose	Cat. No.
CD3	UCHT1	BD Horizon™ BV510	Exclusion (T cell)	<b>563109</b>
CD19	SJ25C1	BD Horizon™ BV510	Exclusion (B cell)	<b>562947</b>
CD20	2H7	BD Horizon™ BV510	Exclusion (B cell)	<b>563067</b>
HLA-DR	G46-6	FITC	DC, NK, monocyte subsets	<b>555811</b>
CD16	3G8	BD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

**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.

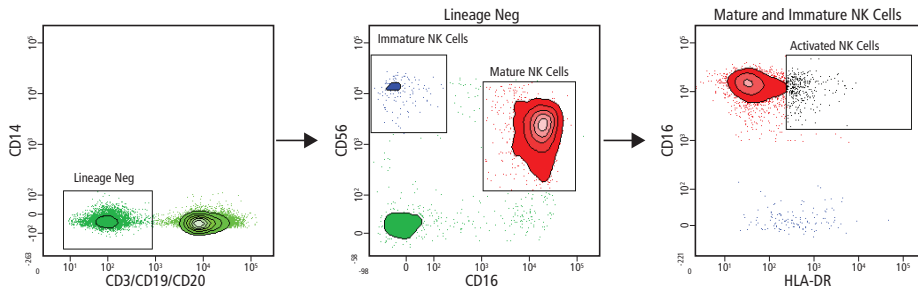
**Data**

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



**Data**

Analysis of Human Natural Killer (NK) Cells



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