BioLegend

The path to legendary discovery

VOL 01

Expanding the Parameters of Investigation: Multicolor Immunofluorescent Staining

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Multicolor Fluorochrome Antibody Conjugates: Expanding the Parameters of Investigation

To identify discrete cell subsets involved in the immune response, it has become essential to examine multiple surface markers in concert with intracellular markers (such as transcription factors or cytokines). For example, the identification of naturally occurring T regulatory cells (T_{reg}) in the peripheral blood has been shown to account for 5–10% of the total $CD4^+$ T cells.[1–3] Because T_{reg} constitute a small percentage of the total lymphocyte population and lack unique cell surface markers, identification of this cell population requires that multicolor analysis of both cell surface and intracellular proteins be carried out. In the case of naturally occurring T_{reg} these might include the cell surface proteins CD4, CD25 (IL-2Rα), GITR, and CD152 (CTLA-4) known to be expressed at high levels on T_{reg} as well as the intracellular transcription factor FoxP3, a known master regulator for T_{reg} development and function.[2–6]

For the simultaneous examination of multiple cell surface coupled with intracellular markers, careful choice of the fluorescent antibody conjugates must be considered. Several parameters must be taken into account when determining the choice of antibody conjugates including the relative expression levels of the antigen of interest, the relative brightness of the dye and the interactions between the various fluorochromes with respect to spectral overlap and compensation. In cases where there is a high density of antigen, a relatively dim dye can be used, whereas a low density antigen requires a brighter dye for accurate measurement. In the case of novel cell surface markers where the expression levels are not well established, it is best to choose brighter fluorochromes and combinations of fluorochromes that have minimal influence on the channels in which the markers of interest are measured. For more detailed accounts of experimental considerations given to multiparametric analysis of rare populations, please see references.[7–9]

Why more colors?

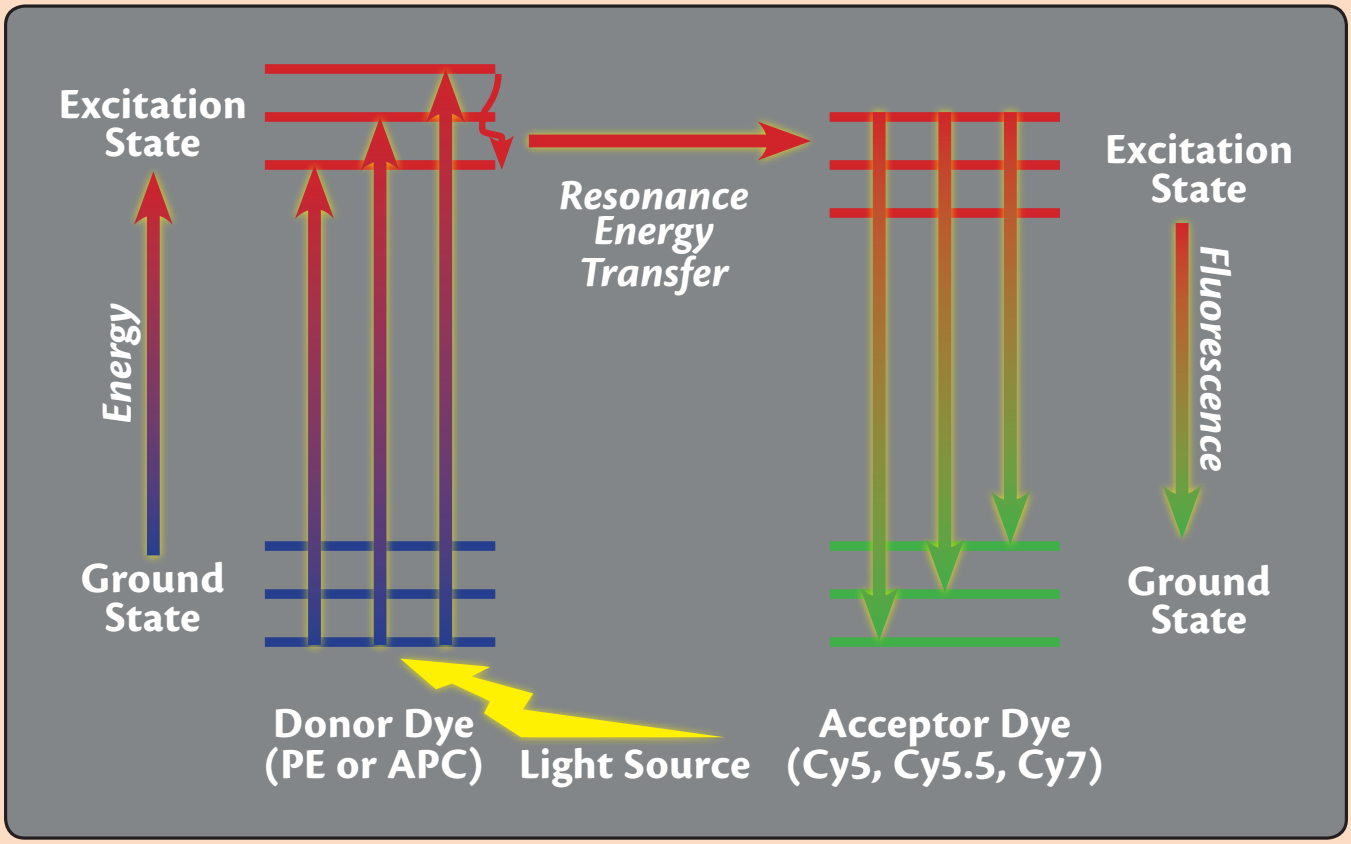
Simply put, more colors give more information about the target cell population and can identify functionally distinct cell populations. Investigators have been able to use 11-color, 13-parameter flow cytometric analyses to identify 75 phenotypically distinct lymphocyte subpopulations.[9] More recently, 17-color and 19-parameter flow cytometric analysis has been successfully performed in Roederer's lab.[10] In order to perform multicolor flow cytometry effectively, instrumentation with multiple lasers, enhanced optics, and increased numbers of PMTs are needed. In addition, antibody options with expanded menus of fluorescent dye conjugates that have multiple excitations/absorptions, spectral separation of emissions, and sufficient "brightness" to detect low density antigens and rare populations must be available. Low molecular weight (<1000 daltons), synthetic dyes such as FITC were among the first generation of dyes used for flow cytometry. Next, the naturally occurring high molecular weight (35–200 kD) phycobiliprotein dyes such as APC and PE were used for antibody conjugation. Antibody conjugates of these dyes have high sensitivity compared to FITC conjugates. A new generation of synthetic dyes such as cyanine and Alexa, and tandem dyes followed which are highly sensitive with increased emission spectra options. Although the cyanine and Alexa dyes offer increased emission spectra options, they can require two or more laser lines for multicolor options. Tandem dyes are especially useful for users with less complex flow cytometry systems as they provide a method to maximize the number of colors using a minimal number of lasers for excitation.

Tandem Dyes

Tandem dyes such as PE/Cy5, PE/Cy5.5, PE/Cy7, APC/Cy5.5 and APC/Cy7 are especially useful for users who have limited laser availability as they provide expanded options for multicolor applications. For example, tandem dye technology can provide 5–6 colors from a 488 excitation source and 3–4 colors from a 633 excitation source. Tandem dyes by definition contain an acceptor dye and a covalently linked donor dye in close proximity to permit energy resonance transfer. When the donor dye (PE or APC) is excited by a laser, energy transfer occurs to the acceptor dye (Cy5, Cy5.5, or Cy7) in the absence of photon emission. The excited donor dye then emits a photon consistent with its return to

Figure 1

Mechanism of Resonance Energy Transfer in Tandem Dyes



Resonance energy transfer in tandem dyes is a process by which a fluorescent molecule such as PE or APC (donor dye) can be excited and transfer its energy to another nearby fluorescent molecule such as Cy5, Cy5.5, or Cy7 (acceptor dye) which then emits fluorescence. The process of fluorescence energy transfer requires that the donor and acceptor dye be in close proximity (30-50 nm) and that the emission spectrum of the donor dye overlap (at least to a small extent) the excitation spectrum of the acceptor dye. The resulting tandem dye can be excited at wavelengths associated with the donor dye with emission spectra shifted to longer wavelengths. In an optimized tandem dye, there is little photon emission associated with the donor dye itself, instead most of the energy is transferred to the acceptor dye which emits fluorescence. The excitation and emission maxima for various tandem dye combinations are shown in Table I.

ground state (Figure 1). The parameters of excitation and emission for fluorescent dye-antibody conjugates (as well as the instrumentation types that will support analysis using various multi-color options) are summarized in Table I (on pages 6 & 7).

Alexa Fluor® and Pacific Blue™ Dyes

Alexa Fluor® dyes are a family of fluorescent dyes that show exceptional fluorescence and photostability compared to other commonly used dyes with similar spectral properties. In addition to these properties, the fluorescence output of Alexa dyes is virtually independent of pH, showing pH insensitivity over a broad range (4–10 pH). The number in each of the Alexa dye names, for example Alexa Fluor® 488, Alexa Fluor® 647 and Alexa Fluor® 700, refers to the approximate excitation maxima of the dyes which nearly matches the principal wavelength of commonly used excitation sources in both flow cytometry and fluorescence microscopy (see Table I). Pacific Blue™ is a relatively new fluorescent dye that also shows exceptional fluorescence and photostability. The individual properties of the Alexa Fluor® and Pacific Blue™ dyes are detailed below.

Alexa Fluor® 488: Has spectral characteristics almost identical to those of fluorescein (FITC or FAM), but with far greater fluorescence per protein conjugate. Alexa Fluor® 488 shows strong absorption giving an extinction coefficient of >71,000$cm^{-1}M^{-1}$. Alexa Fluor® 488 conjugates can be detected with any flow cytometer equipped with an argon laser that emits at 488 nm. Peak emission of Alexa Fluor® 488 (519 nm) is measured in the FL1 channel. The higher fluorescence intensity of Alexa Fluor® 488 conjugates is advantageous in flow cytometric applications where a strong signal is needed to detect receptors or other proteins that are present in small numbers. Alexa Fluor® 488 has a fluorescence lifetime of 4.1 nanoseconds and is much more photostable than fluorescein allowing additional time for image capture and observation, traits especially useful for fluorescence microscopy (including fluorescence polarization measurements and fluorescence correlation spectroscopy).

Alexa Fluor® 647: Has spectral characteristics that can be an alternative to either APC or Cy5 dyes. Spectral characteristics of Alexa Fluor® 647 virtually match those of the Cy5 dye, but with greater total fluorescence per protein conjugate. Alexa Fluor® 647 shows strong absorption giving an extinction coefficient of 239,000$cm^{-1}M^{-1}$. This dye can be excited by long-wavelength excitation sources such as HeNe or red diode lasers emitting at 633 and 635 nm, respectively. Like other Alexa dyes, Alexa Fluor® 647 conjugates are exceptionally bright. Unlike the Cy5 dye, Alexa Fluor® 647 shows very little change in absorbance or fluorescence spectra when conjugated to proteins. Alexa Fluor® 647 is typically three to four times brighter than Cy5 when conjugated to proteins at the same degree of substitution (and sometimes as much as 40-fold brighter at equal antibody concentrations). The higher fluorescence intensity of Alexa Fluor® 647 conjugates (resulting from low fluorescence quenching after protein conjugation) has obvious advantages in multi-color flow cytometric applications where strong signals are required to detect scarce receptors or proteins. Because the photostability of Alexa Fluor® 647 far exceeds that of both APC and Cy5 dyes, this dye is also an obvious choice for many fluorescence microscopy applications.

Alexa Fluor® 700 Dye. The Alexa Fluor® 700 dye fills a need for a bright and photostable conjugate that can be excited by inexpensive, long wave excitation sources such as the red He-Ne (633 nm) and red laser diodes. The Alexa Fluor® 700 dye can be excited by long-wavelength excitation sources such as HeNe or red diode lasers emitting at 633 and 635 nm, respectively. Flow cytometric applications using Alexa Fluor® 700 requires a red-enhanced PMT or other suitable detection system. Because human vision is insensitive to light beyond 650 nm, it is not possible to view this far-red dye through the eyepiece of a conventional fluorescence microscope.

Pacific Blue™ Dye: Pacific Blue™ is based on the 6,8-difluoro-7-hydroxycoumarin fluorophore and exhibits bright blue-fluorescent emission near 460 nm with excellent photostability. Conjugates of the Pacific Blue™ dye are optimally excited by krypton-ion gas lasers or the 405 nm spectral line of the blue diode laser recently developed for fluorescence microscopy and multi-color flow cytometry.

Concluding Remarks

To expand your research options, BioLegend is proud to offer a variety of primary antibodies against mouse, human, and rat markers (as well as isotype control antibodies) conjugated to Alexa Fluor® 488, Alexa Fluor® 647, Alexa Fluor® 700, Pacific Blue™. In addition, BioLegend offers a wide variety of primary antibodies, isotype control antibodies and detection reagents such as Streptavidin conjugated to tandem dyes including PE/Cy5, PE/Cy5.5, PE/Cy7, APC/Cy5.5, and APC/Cy7. Optimized tandem dyes prepared by our experienced staff at BioLegend provides the highest fluorescence intensity with the lowest cross-over ratio and the highest lot-to-lot reproducibility, making publication quality flow cytometric profiles.[11–13] Examples of cell surface staining and intracellular staining using various multicolor antibodies are shown in Figure 2 (on page 8). Please see our website (www.biolegend.com; Support section tab, Technical Protocols) for detailed flow cytometric staining protocols and our expanding colors.

Table I: Fluorochromes for Flow Cytometric Analysis

Fluorochrome	Excitation Max (nm)	Excitation Laser Lines (nm)	Emission Max (nm)	BD FACScan™	BD FACSCalibur™	BD FACSVantage™SE	BD FACSCanto™
Cascade Blue®	377	360, 405, 407	420			Blue	
Alexa Fluor® 405	401	360, 405, 407	421			Blue	
Pacific Blue™	410	360, 405, 407	455			Blue	
Alexa Fluor® 488	495	488	519	Green	Green	Green	Green
FITC (fluorescein)	493	488	525	Green	Green	Green	Green
PE (R-Phycoerythrin)	496, 565	488	575	Yellow	Yellow	Yellow	Yellow
PE/Texas Red®	496, 565	488	613	Orange		Orange	
APC (allophycocyanin)	645	595, 633, 635, 647	660		Red	Red	Red
Cy5	649	633, 635	666		Red	Red	
Alexa Fluor® 647	650	595, 633, 635, 647	668		Red	Red	
PE/Cy5	496, 565	488	670	Red	Red	Red	
PerCP	482	488	675	Red	Red		Red
APC/Cy5.5	650	595, 633, 635, 647	690		Far-Red	Far-Red	Far-Red
PE/Cy5.5	496, 565	488	690	Far-Red	Far-Red	Far-Red	Far-Red
PerCP-Cy5.5	482	488	690	Far-Red	Far-Red	Far-Red	Far-Red
Alexa Fluor® 700	696	633, 635	719			Far-Red	
APC/Cy7	650	595, 633, 635, 647	774			InfraRed	InfraRed
PE/Cy7	496, 565	488	774	InfraRed	InfraRed	InfraRed	InfraRed
Viability Probe							
7-AAD (7-amino-actinomycin D)	546	488	647	Red	Red	Red	Red
PI (Propidium Iodide)	305, 540	325, 360, 488	620	Orange	Orange	Orange	Orange

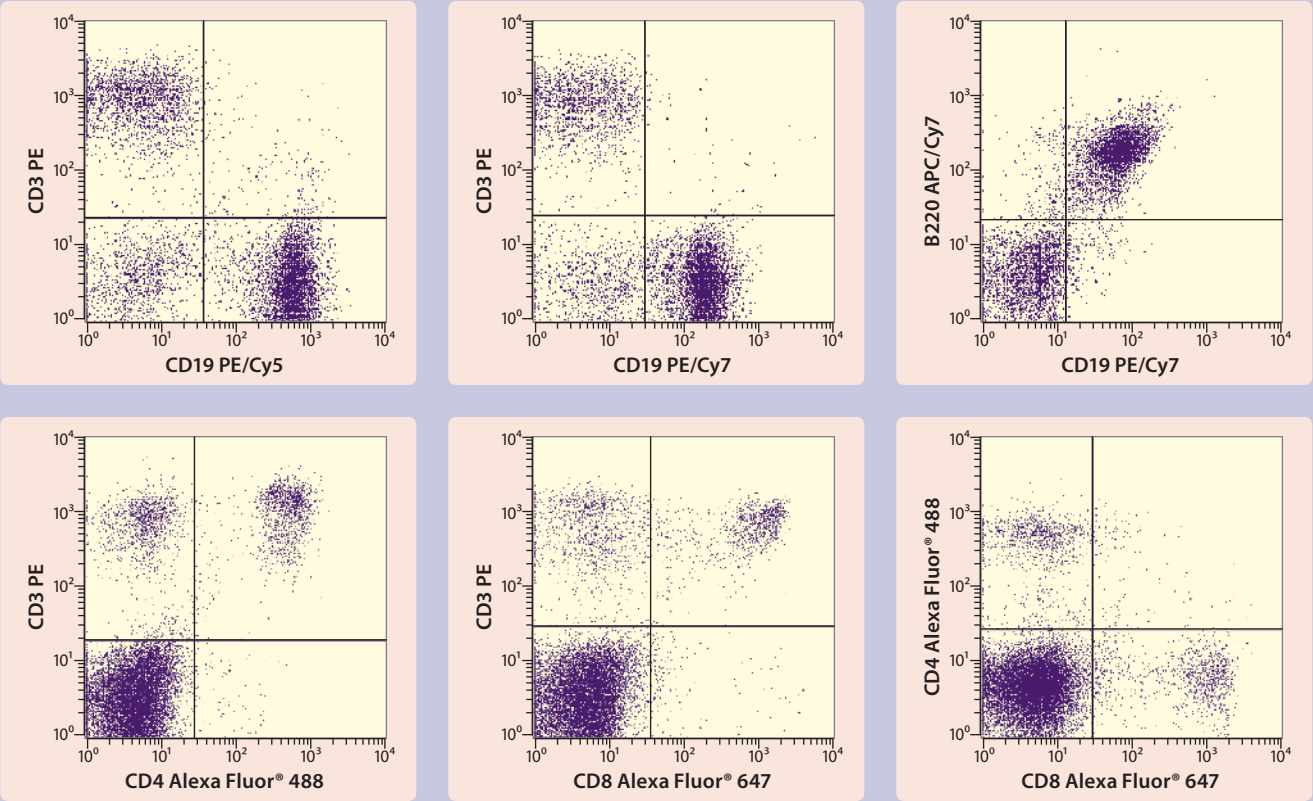
* These fluorochromes can be used as single color configurations or multi-colored configurations depending on instruments, available lasers and filters, and appropriate color compensation settings.

** This chart is provided as general information. User must check instrument capability and choose appropriate setting.

Table I: Fluorochromes for Flow Cytometric Analysis

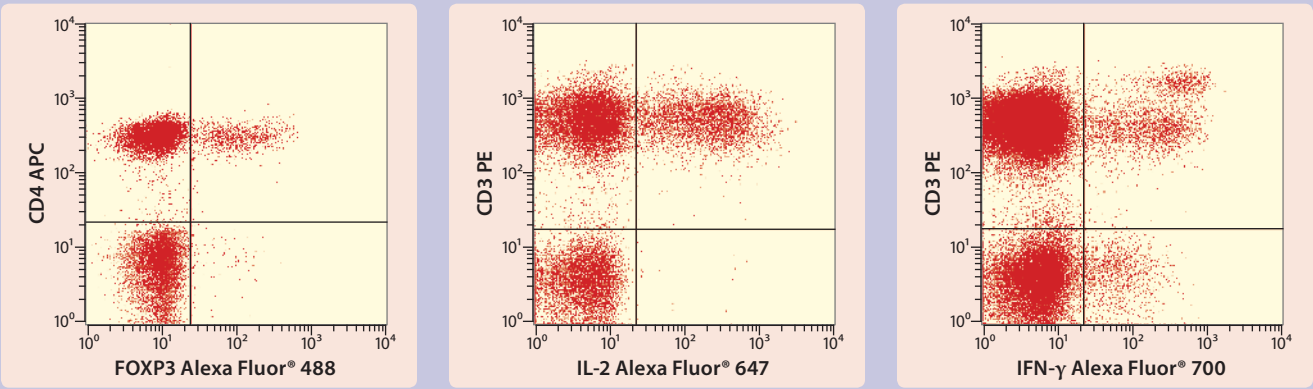
BD™ LSR II	BD FACSAria™	Coulter® EPICS® XL™/XL-MCL™	Beckman Coulter® FC500	CYTOPEIA inFlux™	DakoCytomation CyAn™ ADP	DakoCytomation MoFlo™	Fluorochrome
Blue	Blue			Blue	Blue	Blue	*Cascade Blue®*
Blue	Blue			Blue	Blue	Blue	*Alexa Fluor® 405*
Blue	Blue			Blue	Blue	Blue	*Pacific Blue™*
Green	Green	Green	Green	Green	Green	Green	*Alexa Fluor® 488*
Green	Green	Green	Green	Green	Green	Green	*FITC (fluorescein)*
Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	*PE (R-Phycoerythrin)*
Orange	Orange	Orange	Orange	Orange	Orange	Orange	*PE/Texas Red®*
Red	Red		Red	Red	Red	Red	*APC (allophycocyanin)*
Red	Red		Red	Red	Red	Red	*Cy5*
Red	Red		Red	Red	Red	Red	*Alexa Fluor® 647*
Red	Red	Red	Red	Red	Red	Red	*PE/Cy5*
Red	Red	Red	Red	Red	Red		*PerCP*
Far-Red	Far-Red		Far-Red	Far-Red	Far-Red	Far-Red	*APC/Cy5.5*
Far-Red	Far-Red	Far-Red	Far-Red	Far-Red	Far-Red	Far-Red	*PE/Cy5.5*
Far-Red	Far-Red	Far-Red	Far-Red	Far-Red	Far-Red		*PerCP-Cy5.5*
Far-Red	Far-Red			Far-Red	Far-Red	Far-Red	*Alexa Fluor® 700*
InfraRed	InfraRed			InfraRed	InfraRed	InfraRed	*APC/Cy7*
InfraRed	InfraRed	InfraRed	InfraRed	InfraRed	InfraRed	InfraRed	*PE/Cy7*
							Viability Probe
Red	Red	Red	Red	Red	Red	Red	*7-AAD (7-amino-actinomycin D)*
Orange	Orange	Orange	Orange	Orange	Orange	Orange	*PI (Propidium Iodide)*

Figure 2a
Mouse Cell Surface Antigens Detected with Multicolor Antibody Conjugates



BALB/c mouse splenocytes were stained with various antibodies including CD3 PE (Cat. No. 100205) and CD19 PE/Cy5 (Cat. No. 115509) top left panel; CD3 PE (Cat. No. 100205) and CD19 PE/Cy7 (Cat. No. 115519) top middle panel; and B220 APC/Cy7 (Cat. No. 103223) and CD19 PE/Cy7 (Cat. No. 11519) top right panel. Staining is shown in the bottom row using CD3 PE (Cat. No. 100205) and CD4 Alexa Fluor® 488 (Cat. No. 100425) left panel; CD3 PE (Cat. No. 100205) and CD8 Alexa Fluor® 647 (Cat. No. 100727) middle panel; and CD4 Alexa Fluor® 488 (Cat. No. 100425) and CD8 Alexa Fluor® 647 (Cat. No. 100727) right panel.

Figure 2b
Human Intracellular Staining of Transcription Factors and Cytokines with Cell Surface Markers Using Multicolor Antibody Conjugates



Human peripheral blood mononuclear cells (PBMCs) were stained with CD4 APC (Cat. No. 300513) and FOXP3 Alexa Fluor® 488 (Cat. No. 320211) as shown in left panel; PMA and ionomycin stimulated PBMCs were stained with CD3 PE (Cat. No. 300407) and IL-2 Alexa Fluor® 647 (Cat. No. 500317) as shown in middle panel, and with CD3 PE (Cat. No. 300407) and IFN-γ Alexa Fluor® 700 (Cat. No.502519) as shown in right panel.

BioLegend Fluorochrome Conjugated Antibodies

Specificity	Clone	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
Mouse CDs and Related Molecules													
CD2	RM2-5	X	X										
CD3	17A2	X	X							X	X	X	X
CD3ε	145-2C11	X	X	X	X	X	X	X		X	X		
CD4	GK1.5	X	X	X	X	X	X	X	X	X	X	X	X
CD4	RM4-5	X	X	X	X	X	X	X	X	X	X	X	X
CD4	RM4-4	X	X										
CD5	53-7.3	X	X	X						X	X		
CD8a	53-6.7	X	X	X	X	X	X	X	X	X	X	X	X
CD8a	5H10-1	X											
CD8b	CD8b	X	X										
CD11a (Integrin α_L)	2D7	X	X										
CD11a (Integrin α_L)	M17/4	X	X							X	X		
CD11b (Integrin α_M)	M1/70	X	X	X		X	X			X	X	X	
CD11c (Integrin α_X)	N418	X	X	X		X	X			X	X	X	
CD16/32 (Fcγ receptor III/II)	93	X	X							X	X	X	
CD18 (Integrin β_2)	M18/2	X	X							X	X		
CD19	MB19-1	X											
CD19	6D5	X	X	X	X	X	X	X		X	X	X	X
CD23 (Fcε RII)	B3B4	X	X							X	X		
CD24 (HSA)	M1/69	X	X	X			X			X	X		
CD25 (IL-2Rα)	3C7		X										
CD25 (IL-2Rα)	PC61	X	X	X		X	X			X	X	X	X
CD28	37.51		X	X			X						
CD29 (Integrin β_1)	HMß1-1	X	X				X			X	X	X	
CD30	mCD30.1		X										
CD31 (PECAM-1)	390	X	X				X			X	X		
CD31 (PECAM-1)	MEC13.3	X	X				X			X	X		
CD34	MEC14.7		X										
CD36	HM36		X							X	X		
CD38	90	X	X	X			X			X	X		
CD40	1C10		X	X									
CD40	HM40-3	X								X	X		
CD43 Activation-Associated Glycoform	1B11	X	X										
CD44	IM7	X	X	X			X			X	X	X	X
CD45 (Ly-5)	30-F11	X	X	X	X	X	X		X	X	X	X	X
CD45.1 (Ly-5.1)	A20	X	X		X		X	X	X	X	X	X	X
CD45.2 (Ly-5.2)	104	X	X		X		X	X		X	X	X	X
CD45R (B220)	RA3-6B2	X	X	X	X	X	X	X	X	X	X	X	X

Specificity	Clone	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
Mouse CDs and Related Molecules *(continued)*													
CD45RB	C363-16A	X	X										
CD48	HM48-1	X	X										
CD49b/Pan-NK Cells (DX5)	DX5	X	X				X			X	X		
CD49b (Integrin α_2, VLA-2α)	HMα2	X	X							X	X		
CD49d (Integrin α_4, VLA-4α)	R1-2	X	X							X	X		
CD49d (Integrin α_4, VLA-4α)	9C10(MFR4.B)		X										
CD49e (Integrin α_5, VLA-5α)	5H10-27(MFR5)		X							X	X		
CD49e (Integrin α_5, VLA-5α)	HMα5-1		X										
CD49f (Integrin α_6, VLA-6α)	GoH3	X								X	X		
CD51 (Integrin α_v)	RMV-7		X										
CD54 (ICAM-1)	YN1/1.7.4	X	X							X	X	X	X
CD61 (Integrin β_3)	2C9.G2 (HMß3-1)	X	X							X	X		
CD62L (L-selectin)	MEL-14	X	X	X		X	X			X	X	X	X
CD69 (VEA)	H1.2F3	X	X	X		X	X			X	X		
CD70	FR70		X										
CD71 (Trf R)	RI7217	X	X										
CD80 (B7-1)	16-10A1	X	X	X			X			X	X		
CD81 (TAPA-1)	Eat-2		X										
CD86 (B7-2)	GL-1	X	X	X		X	X			X	X	X	X
CD86 (B7-2)	PO3	X	X	X		X	X			X	X	X	
CD90.1 (Thy-1.1)	OX-7	X								X	X		
CD90.2 (Thy-1.2)	30-H12	X	X	X			X			X	X	X	
CD94 (Kp43)	18d3	X	X										
CD102 (ICAM-2)	3C4 (MIC2/4)	X								X	X		
CD105 (Endoglin)	MJ7/18									X			
CD106 (VCAM-1)	429 (MVCAM.A)	X								X	X		
CD107b (Mac-3)	M3/84	X	X							X	X		
CD117 (c-Kit)	2B8	X	X	X		X	X			X	X		X
IFN-γR β chain	MOB-47		X										
CD120a (TNF R I/p55)	55R-286		X										
CD120b (TNF R II/p75)	TR75-89		X										
CD121a (IL-1 R I/p80)	JAMA-147		X										
CD122 (IL-2Rβ)	5H4		X										
CD123 (IL-3Rα)	5B11		X										
CD126 (IL-6Rα)	D7715A7		X										
CD127 (IL-7Rα)	SB/199	X	X							X	X		
CDw137 (4-1BB)	17B5		X										
CDw137L (4-1BB Ligand)	TKS-1		X										
CD150 (SLAM)	TC15-12F12.2		X	X		X	X			X	X		
CD152 (CTLA-4)	UC10-4B9		X										
CD153 (CD30 Ligand)	RM153		X										
CD154 (CD40 Ligand)	MR1		X										

Specificity	Clone	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
CD178 (Fas Ligand)	MFL3		X										
CD178.1 (Fas Ligand)	Kay-10		X										
CD180 (RP105)	RP/14		X										
CD195 (CCR5)	HM-CCR5		X							X			
CD197 (CCR7)	4B12		X	X			X			X	X		
CD210 (IL-10 R)	1B1.3a		X										
CD252 (OX40 Ligand)	RM134L		X										
CD253 (TRAIL)	N2B2		X										
CD254 (TRANCE, RANKL)	IK22/5		X										
CD255 (TWEAK)	MTW-1		X										
CD262 (DR5, TRAIL-R2)	MD5-1		X										
CD265 (RANK)	R12-31		X										
CD266 (TWEAK Receptor, Fn14)	ITEM-4		X										
CD273 (B7-DC, PD-L2, PDL2, B7DC)	TY25		X										
CD275 (B7-RP1, ICOSL, B7H2)	HK5.3		X										
CD278 (ICOS)	15F9		X	X									
CD278 (ICOS)	C398.4A	X	X				X			X	X		
CD279 (PD-1)	RMP1-30		X										
CD284 (TLR4 /MD2 Complex)	MTS510		X										
CD314 (NKG2D)	A10		X										
CD314 (NKG2D)	C7	X	X										
Cytochrome c	6H2.B4	X											
FOXP3	150D		X							X	X		
FOXP3 Flow Kit	150D		X							X	X		
Mouse Treg Flow Kit	150D									X			
GITR	YGITR 765	X	X	X		X	X			X	X	X	
GITRL	YGL 386		X										
IgD	11-26c.2a	X	X										
IgD^a (Igh-5a)	AMS-9.1	X											
IgM	RMM-1	X	X				X						
IgM^a (Igh-6a)	DS-1	X	X										
IgM^b (Igh-6b)	AF6-78	X	X										
Integrin β_7	LS722	X	X										
LPAM-1 (Integrin $\alpha_4\beta_7$)	DATK32		X										
Ly-6A/E (Sca-1)	D7	X	X	X		X	X			X	X		X
Ly-6G/Ly-6C (Gr-1)	RB6-8C5	X	X	X		X	X			X	X	X	
Ly-49A	YE1/48.10.6	X	X										
Ly-49C/F/I/H	14B11	X	X										
Ly-51 (BP-1)	6C3	X	X							X	X		
MAdCAM-1	MECA-367									X			
MD-1	MD-113		X										
MD-1	MD-14		X										
NK-1.1	PK136	X	X	X		X	X			X	X		X

Specificity	Clone	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
Mouse CDs and Related Molecules *(continued)*													
NOTCH1	mN1A		X										
Panendothelial Cell Antigen	MECA-32									X			
TER-119/Erythroid Cells	TER-119	X	X	X			X			X	X	X	
Tim-1	RMT1-4		X										
Tim-3	RMT3-23		X										
ZAP-70	1E7.2		X										
Mouse MHC Antigens													
H-2D^b	KH95	X	X							X	X		
H-2D^d	34-2-12	X	X							X	X		
H-2D^k	15-5-5	X	X										
H-2K^b	AF6-88.5	X	X							X	X		
H-2K^b/H-2D^b	28-8-6	X	X							X	X		
H-2K^b/H-2D^d	34-1-2S	X	X							X	X		
H-2K^d	SF1-1.1	X	X							X	X		
H-2K^k	AF3-12.1	X											
H-2K^k	36-7-5	X	X							X	X		
H-2K^q	KH114	X											
H-2L^d/H-2D^b	28-14-8	X	X							X	X		
I-A/I-E	M5/114.15.2	X	X	X			X			X	X	X	X
I-A^b	AF6-120.1	X	X							X	X		
I-A^b	KH74	X								X	X		
I-A^b (Aβ^b)	25-9-17	X								X	X		
I-A^d	39-10-8	X								X	X		
I-A^k (Aα^k)	11-5.2	X	X							X	X		
I-A^k (Aβ^k)	10-3.6	X	X							X	X		
I-A^q	KH116									X	X		
I-E^k	14-4-4S	X	X							X	X		
Mouse T Cell Receptors *(TCRs)*													
β T Cell Receptor	H57-597	X	X	X			X			X	X		
γ/δ T Cell Receptor	UC7-13D5	X	X							X	X		
γ/δ T Cell Receptor	GL3	X	X										
Vβ7 T Cell Receptor	TR310	X	X										
Vβ 8.3 T Cell Receptor	8C1	X	X										
Mouse Cytokines/Chemokines													
IL-1α	ALF-161		X										
IL-2	JES6-5H4	X	X				X			X	X	X	
IL-3	MP2-8F8		X										
IL-4	11B11		X				X			X	X		
IL-5	TRFK5		X				X						
IL-6	MP5-20F3		X										
IL-10	JES5-16E3	X	X				X			X	X		

Specificity	Clone	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
IL-12	C15.6		X				X						
IL-17	TC11-18H10.1		X										
GM-CSF	MP1-22E9	X	X										
IFN-γ	DB-1	X	X										
IFN-γ	XMG1.2	X	X				X			X	X	X	
MCP-1	2H5		X										
TNF-α	MP6-XT22	X	X				X			X	X	X	
TNF-α	TN3-19.12		X										
TRANCE	IK22/5		X										
Rat CDs and Related Molecules													
CD2	OX-34	X	X										
CD3	1F4	X											
CD4	W3/25	X	X				X						
CD5	R1-3B3	X											
CD5	OX-8	X	X										
CD8a	G28	X	X				X						
CD8b	341	X											
CD11b/c (Integrin α_M/α_X)	OX-42	X	X				X						
CD25 (IL-2Rα)	OX-39	X	X										
CD28	JJ319	X	X				X						
CD29 (Integrin β_1)	HMß1-1	X	X				X			X	X	X	
CD45 (LCA)	OX-1	X	X										
CD45RA	OX-33	X	X										
CD49d (Integrin α_4)	MRα4-1	X											
CD49e (Integrin α_5)	HMα5-1		X										
CD61 (Integrin β_3)	2C9.G2 (HMß3-1)	X	X							X	X		
CD80 (B7-1)	3H5		X										
CD81 (TAPA-1)	Eat-2		X										
CD86 (B7-2)	24F	X	X										
CD90.1 (Thy-1.1)	OX-7	X								X	X		
CD106 (VCAM-1)	MR106		X										
CD126 (IL-6Rα)	D7715A7		X										
CD252 (OX-40 Ligand)	ATM-2		X										
CD278 (ICOS)	C398.4A	X	X				X			X	X		
FOXP3	150D		X							X	X		
FOXP3 Flow Kit	150D		X							X	X		
IgM	MRM-72	X											
RT1D	14-4-4S	X	X							X	X		
Rat T Cell Receptors (TCRs)													
α/β T Cell Receptor	R73	X	X				X						
γ/δ T Cell Receptor	V65	X	X										
Vβ 8.2/8.4 T Cell Receptor	R78	X											

Specificity	Clone	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
Human CDs and Related Molecules													
CD1a (T6)	HI149	X	X	X			X			X	X	X	X
CD2 (LFA-2)	TS1/8	X	X	X									
CD2 (LFA-2)	RPA-2.10	X	X	X									
CD3 (T3)	HIT3a	X	X	X		X	X		X	X	X	X	
CD3 (T3)	UCHT1	X	X	X		X	X			X	X	X	X
CD4 (T4)	RPA-T4	X	X	X		X	X		X	X	X	X	X
CD5	UCHT2	X	X	X			X			X	X		
CD6	MEM-98	X	X										
CD6	BL-CD6	X	X										
CD7	MEM-186	X	X	X									
CD8a (T8)	HIT8a	X	X	X		X	X					X	
CD8a (T8)	RPA-T8	X	X	X	X	X	X		X	X	X	X	X
CD9	HI9a	X	X										
CD10 (CALLA)	MEM-78		X	X									
CD10 (CALLA)	HI10a	X	X	X			X						
CD11a (Integrin α_L)	HI111	X	X	X			X			X	X		
CD11b (activated) (Integrin α_M, Mac-1)	CBRM1/5	X	X										
CD11b (Integrin α_M, Mac-1)	ICRF44		X	X			X			X	X		X
CD11c (Integrin α_X)	3.9	X	X	X		X	X			X	X		
CD13 (gp150, APN)	WM15		X				X						
CD14 (LPS Receptor)	M5E2	X	X			X	X		X	X	X	X	X
CD15 (Lewis X)	HI98	X	X				X			X	X		
CD16 (FcγRIII)	3G8	X	X	X		X	X		X	X	X	X	X
CD18 (Integrin β_2)	TS1/18	X	X	X									
CD19 (B4)	HIB19	X	X	X		X	X		X	X	X	X	X
CD20 (Bp35, B1)	2H7	X	X	X		X	X		X	X	X	X	X
CD21 (CR2, C3dR)	LT21	X	X										
CD22 (Siglec-2)	HIB22	X	X	X									
CD24 (HSA)	ML5	X	X							X	X		
CD25 (IL-2Rα)	BC96	X	X	X		X	X		X	X	X	X	X
CD26	BA5b	X	X	X									
CD27	O323	X	X				X				X	X	
CD28 (T44, Tp44)	CD28.2	X	X	X			X			X	X	X	
CD29 (Integrin β_1)	TS2/16		X	X			X		X	X	X	X	
CD31 (PECAM-1)	WM59	X	X							X	X		
CD32 (FcγRII)	FUN-2	X	X				X			X	X		
CD33 (Siglec-3)	HIM3-4	X											
CD33 (Siglec-3)	WM53		X	X			X						
CD36 (gpIIIb, gpIV)	TR9	X	X				X						
CD38 (T10)	HIT2	X	X	X			X			X	X		
CD40 (BP50)	HB14	X	X				X						
CD41 (gpIIb)	HIP8	X	X	X			X						

Specificity	Clone	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
CD42b (gpIbα)	HIP1	X	X										
CD43 (Leukosialin)	MEM-59	X											
CD44 (Hermes, Pgp-1)	IM7	X	X	X			X			X	X	X	X
CD45 (B220, T200, LCA)	HI30	X	X	X		X	X		X	X	X	X	X
CD45RA	HI100	X	X	X			X			X	X	X	X
CD45RB	MEM-55		X										
CD45RO	UCHL1	X	X	X			X			X	X	X	X
CD46	MEM-258	X											
CD48	MEM-102	X											
CD49b (Integrin α_2, VLA-2α)	AK-7	X								X	X		
CD49d (Integrin α_4, VLA-4α)	9F10		X	X			X						
CD49f (Integrin α_6, VLA-6α)	GoH3	X								X	X		
CD50 (ICAM-3)	MEM-171	X	X										
CD51/61 (Integrin α_V/β_3)	23C6	X	X							X	X		
CD52 (CAMPATH-1)	HI186	X											
CD53 (OX44)	MEM-53	X											
CD54 (ICAM-1)	MEM-111	X	X										
CD55 (DAF)	JS11	X	X	X			X						
CD56 (NCAM)	MEM-188	X	X	X			X			X	X	X	X
CD59 (Protectin, H19)	p282 (H19)	X	X										
CD62L (L-Selectin)	DREG-56	X	X	X			X		X	X	X	X	
CD62P (P-Selectin)	AK4	X	X	X			X			X	X		
CD63 (LIMP)	MEM-259	X	X										
CD64 (FcγRI)	10.1	X	X							X	X		
CD66b (CD67)	G10F5	X											
CD69 (VEA)	FN50	X	X	X		X	X		X	X	X	X	X
CD71 (TfR)	MEM-75	X	X										
CD72	3F3	X											
CD80 (B7-1)	2D10	X	X	X						X	X		
CD83 (HB15)	HB15e	X	X	X			X			X	X		
CD86 (B7-2)	IT2.2		X	X			X			X	X		X
CD94	DX22	X	X				X			X	X		
CD94	DX2	X	X	X			X			X	X		
CD98 (4F2)	MEM-108	X											
CD105 (Endoglin)	MEM-226	X											
CD106 (VCAM-1)	STA		X	X			X						
CD108 (H-SEMA, SEMAL)	MEM-150		X										
CD116 (GM-CSFRα)	4H1	X	X										
CD117 (c-kit)	104D2		X				X						
CDw119 (IFN-γRα)	GIR-208		X										
CDw119 (IFN-γRα)	GIR-94		X										
IFN-γ R β chain	2HUB-159		X										
CD123 (IL-3Rα)	6H6		X	X		X	X						

Specificity	Clone	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
Human CDs and Related Molecules *(continued)*													
CD129 (IL-9R)	AH9R7		X										
CD129 (IL-9R)	AH9R2		X										
CDw131 (IL-3R common β)	1C1		X										
CD135 (Flt-3/Flk-2)	BV10A4H2		X				X						
CDw137 (4-1BB)	4B4-1		X										
CDw137L (4-1BBL)	5F4		X										
CD147 (Neurothelin)	HIM6	X								X	X		
CD150 (SLAM)	A12 (7D4)	X	X							X	X		
CD154 (CD40 Ligand)	24-31	X	X	X			X			X	X	X	X
CD158b (KIR2DL2/L3, NKAT2)	DX27	X	X										
CD158e1 (KIR3DL1, NKB1)	DX9	X	X										
CD177	MEM-166	X											
CD178 (FasL, CD95L)	NOK-1		X										
CD180 (RP105)	MHR73-11		X										
CD181 (CXCR1)	8F1/CXCR1	X	X	X			X			X	X		
CD182 (CXCR2)	5E8/CXCR2	X	X	X			X			X	X		
CD184 (CXCR4, Fusin)	12G5		X	X			X						
CD195 (CCR5)	HEK/1/85a	X	X							X	X	X	
CD195 (CCR5)	T21/8		X										
CD195 (CCR5) Phosphorylated (Ser337)	V14/2		X										
CD195 (CCR5) Phosphorylated (Ser349)	E11/19		X										
CD206 (MMR)	15-2	X	X	X			X			X	X		
CD210 (IL-10R)	3F9		X										
CDw218a (IL-18Rα)	H44		X										
CD222	MEM-238	X											
CD235ab (Glycophorin A/B)	HIR2		X	X			X						
CD253 (TRAIL)	RIK-2		X										
CD255 (TWEAK)	CARL-1		X										
CD255 (TWEAK)	CARL-2		X										
DR3 (TRAMP)	JD3		X										
CD261 (DR4, TRAIL-R1)	DJR1		X										
CD262 (DR5, TRAIL-R2)	DJR2-4 (7-8)		X										
CD263 (DcR1, TRAIL-R3)	DJR3		X										
CD264 (DcR2, TRAIL-R4)	DJR4-1		X										
CD264 (DcR2, TRAIL-R4)	DJR4-2		X										
CD266 (Fn14/TWEAK Receptor)	ITEM-1		X										
CD266 (Fn14/TWEAK Receptor)	ITEM-4		X										
CD275 (B7-H2, ICOSL)	2D3		X										
CD278 (ICOS)	C398.4A	X	X				X			X	X		
CD282 (TLR2, Toll Like Receptor 2)	TL2.1	X	X							X	X		
CD283 (TLR3, Toll Like Receptor 3)	TLR-104		X										
CD284 (TLR4, Toll Like Receptor 4)	HTA125		X										

Specificity	Clone	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
CD314 (NKG2D)	1D11		X				X						
β2-microglobulin	2M2	X	X										
CMKLR1	BZ332	X	X										
Cutaneous Lymphocyte-associated Antigen (CLA)	HECA-452	X											
DcR3	3H5/DcR3		X										
FOXP3	206D	X	X							X	X		X
FOXP3	259D		X							X	X		
FOXP3	150D		X							X	X		
FOXP3 Flow Kit	206D		X							X	X		
FOXP3 Flow Kit	259D		X							X	X		
Human Treg Flow Kit	259D									X			
GITR (AITR)	621		X										
GITR Ligand (AITRL)	EB11-2		X										
Granzyme A	CB9	X	X									X	X
IgM	MHM-88	X	X				X						
Integrin β_7	FIB504		X	X			X						
Lymphotoxin β Receptor (LTβR)	31G4D8		X										
MICA/MICB	6D4		X				X			X	X		
NF-κB p50	4D1									X			
NOTCH1	mN1A		X										
PCNA (Proliferating Cell Nuclear Antigen)	PC10	X	X							X	X		
Perforin	dG9	X	X							X	X		
Phosphotyrosine	PY20									X	X		
PLK-1 Phosphorylated (Thr210)	2A3		X							X			
TREM-1	TREM-26		X										
ZAP-70	1E7.2		X										
Human MHC Antigens													
HLA-A,B,C (MHC Class I)	W6/32	X	X	X			X			X	X		X
HLA-DR (MHC Class II)	L243	X	X	X		X	X		X	X	X	X	X
Human T Cell Receptors (TCRs)													
α/β T Cell Receptor (α/β TCR)	IP26	X	X	X						X	X		
α/β T Cell Receptor (α/β TCR)	T10B9	X											
TCR Vβ5 related subset	MEM-262	X								X	X		
Multicolor Cocktail Reagents													
Human CD3 FITC/CD4 PE Cocktail	UCHT1/RPA-T4	X	X										
Human CD3 FITC/CD8 PE Cocktail	UCHT1/RPA-T8	X	X										
Human CD3 FITC/CD19 PE Cocktail	UCHT1/HIB19	X	X										
Human CD3 FITC/(CD16+CD56) PE Cocktail	UCHT1/3G8+MEM-188	X	X										
Human CD3 FITC/HLA-DR PE Cocktail	UCHT1/L243	X	X										
Human CD3 PE-Cy5/CD4 PE/CD8 FITC Cocktail	UCHT1/RPA-T4/RPA-T8	X	X	X									
Human CD4 PE-Cy5/CD25 PE Cocktail	RPA-T4/BC96		X	X									
Human CD45 FITC/CD14 PE Cocktail	HI30/M5E2	X	X										

Specificity	Clone	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
Multicolor Cocktail Reagents *(continued)*													
Mouse IgG1 FITC/IgG1 PE Cocktail	MOPC-21/MOPC-21	X	X										
Mouse IgG1 FITC/IgG2a PE Cocktail	MOPC-21/MOPC-173	X	X										
Mouse IgG1 FITC/IgG1 PE/IgG1 PE-Cy5 Cocktail	MOPC-21/MOPC-21/ MOPC-21	X	X	X									
Human Cytokines/Chemokines													
IL-1α	364-3B3-14		X										
IL-1β	JK1B-1	X											
IL-2	MQ1-17H12	X	X				X			X	X	X	
IL-3	BVD3-1F9		X										
IL-4	8D4-8		X										
IL-4	MP4-25D2	X	X				X			X	X		
IL-5	JES1-39D10		X										
IL-6	MQ2-13A5	X	X				X						
IL-8	JK8-1	X											
IL-9	MH9A4		X										
IL-10	JES3-19F1		X				X						
IL-10	JES3-9D7		X				X			X	X		
IL-12	C11.5	X	X				X						
IL-13	JES10-5A2		X				X						
GM-CSF	BVD2-21C11		X										
IFN-γ	4S.B3	X	X				X			X	X	X	
IFN-γ	B27	X	X				X					X	
MCP-1	5D3-F7		X										
TNF-α	MAb11	X	X				X			X	X	X	
TNF-β	359-81-11		X										
Isotype Controls													
Mouse IgG1, κ	MOPC-21	X	X	X			X		X				
Mouse IgG1, κ	MOPC-21	X	X	X		X	X		X	X	X	X	X
Mouse IgG2a, κ	MOPC-173	X	X	X	X	X	X	X	X	X	X	X	X
Mouse IgG2b, κ	MPC-11	X	X	X		X	X		X	X	X	X	X
Rat IgG1, κ	RTK2071	X	X	X		X	X			X	X	X	X
Rat IgG2a, κ	RTK2758	X	X	X	X	X	X	X	X	X	X	X	X
Rat IgG2b, κ	RTK4530	X	X	X	X	X	X	X	X	X	X	X	X
Rat IgG2c, κ	RTK4174	X	X										
Rat IgM, κ	RTK2118	X	X				X						
Armenian Hamster IgG	HTK888	X	X	X	X	X	X	X		X	X	X	X
Armenian Hamster IgM	HTK204	X								X	X	X	
Mouse IgM	MOPC-104E	X	X				X						
Mouse IgG2b, κ	MG2b-57	X	X				X						
Mouse IgG3, k	MG3-35	X	X										
Mouse IgM, κ	MM-30	X	X				X			X	X	X	X
Syrian Hamster IgG	SHG-1	X	X	X			X						

Specificity	Clone	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
Ig/2nd Step Reagents													
Hamster IgG (Armenian)	Poly4055	X											
Hamster IgG (Syrian)	Poly4056	X											
Human Ig light chain κ	MHK-49	X	X				X			X	X		
Human Ig light chain λ	MHL-38	X	X				X			X	X		
Human IgM	MHM-88	X	X				X						
Mouse IgD	11-26c.2a	X	X										
Mouse IgD^a (Igh-5a)	AMS-9.1	X											
Mouse IgE	RME-1	X	X										
Mouse IgG1	RMG1-1	X											
Mouse IgG2a	RMG2a-62	X											
Mouse IgG2b	RMG2b-1	X											
Mouse IgM	RMM-1	X	X				X						
Mouse IgM^a (Igh-6a)	DS-1	X	X										
Mouse IgM^b (Igh-6b)	AF6-78	X	X										
Mouse IgG	Poly4053	X	X				X						
Mouse IgG	Poly4060	X											
Rat IgG1	MRG1-58	X											
Rat IgG2a	MRG2a-83	X											
RatIgG2b	MRG2b-85	X											
Rat IgM	MRM-72	X											
Rat IgG	Poly4054	X	X				X						
Avidin		X											
Streptavidin		X	X	X		X	X		X				

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