Antibodies to Human Cell-Surface Markers Tested for BD Phosflow Protocols

Many cell-surface antigens are sensitive to fixation/permeabilization procedures, resulting in loss of recognition by their respective antibodies. For your information, BD Biosciences has tested many antibodies conjugated to various fluorochromes under several fixation/permeabilization conditions, and the results are summarized as follows.

			Protocol I *Detergent method*	Protocol II *Mild alcohol method*	Protocol III *Harsh alcohol method*	Protocol IV *Detergent method*
Fix buffer recommended for PBMCs or cell lines:			BD Cytofix™ Buffer (554655)	BD Cytofix™ Buffer (554655)	BD Cytofix™ Buffer (554655)	BD Cytofix™ Buffer (554655)
Fix buffer recommended for whole blood, splenocytes, bone marrows, and other sample types containing erythrocytes:			BD™ Phosflow Lyse/Fix Buffer (558049)	BD™ Phosflow Lyse/Fix Buffer (558049)	BD™ Phosflow Lyse/Fix Buffer (558049)	BD™ Phosflow Lyse/Fix Buffer (558049)
Perm Buffer:			BD™ Phosflow Perm/Wash Buffer I (557885)	BD™ Phosflow Perm Buffer II (558052)	BD™ Phosflow Perm Buffer III (558050)	BD™ Phosflow Perm Buffer IV (560746)
Specificity	**Clone**	**Fluorochrome**				
Human CD3	SK7	APC	+	+	+	+
		APC-Cy™7	+	+	–	+
		FITC	+	+	+	+
		PE	+	+	+	+
		PE-Cy™7	+	+	+	+
		PerCP	+	+	+/–	–
		PerCP-Cy™5.5	+	+	+	+
	UCHT1	Alexa Fluor® 488	+	+	+	+
		Alexa Fluor® 647	+	+	+	+
		Alexa Fluor® 700		+	+/–	–
		APC	+	+	+	+
		FITC	+	+	+	+
		BD Horizon™ V450			+	+
		Pacific Blue™		+	+	+
		PE	+	+	+	+
		PE-Cy™5	+	+	+	+
		PE-Cy7	+	+	+	+
	HIT3a	APC	+	–	–	+/–
		FITC		–	–	
		PE	+	–	–	–
		PE-Cy5	+		–	–
	SP34	PE-Cy7	+		–	+/–
		PerCP	+	+	+	–
Human CD4	L200	FITC	+	+	+	
		PE	+	+	+	–
		PE-Cy7	+	+	+	–
		PerCP	+	+	+	–
		PerCP-Cy5.5	+	+	+	
	RPA-T4	Alexa Fluor® 488	+	+	+	–
		Alexa Fluor® 647	+	+	+	–
		Alexa Fluor® 700	+		–	–
		APC	+	+	+	+
		APC-Cy7	+	–	–	–
		FITC	+	+	+	–
		Pacific Blue™	+	+	+	–
		PE	+	+	+	+
		PE-Cy5	+		+	–
		PE-Cy7	+	+	+	–
	SK3	AmCyan	–	–	–	–
		APC	+	+	+	+
		APC-Cy7	+/–	–	–	–
		FITC	+	+	+	+
		PE	+	+	+	+
		PE-Cy7	+	+	+	
		PerCP	+	+	+	–
		PerCP-Cy5.5	+	+	+	+
	L120	PE	–	–	–	–
Human CD5	UCHT2	PE	+	+	+	
		APC	+	+/–	–	

Notes:
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Antibodies to Human Cell-Surface Markers Tested for BD Phosflow Protocols *(continued)*

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Specificity	**Clone**	**Fluorochrome**				
Human CD8	HIT8a	APC-Cy7	+	+	–	+
		FITC	+	+	+	+
		PE	+	+	+	+
		PE-Cy5	+		+	+
	RPA-T8	Alexa Fluor® 488	+	+	+	+
		Alexa Fluor® 647	+	+	+	+
		Alexa Fluor® 700		+		
		APC	+	+	+	+
		APC-Cy7		+		
		FITC	+	+	+	+
		Pacific Blue™		+	+	
		PE	+	+	+	+
		PE-Cy5	+		+	
		PE-Cy5	+	+	+	+
		PE-Cy7	+	+	–	+
	SK1	APC	+	+	+	+
		APC-Cy7	+/–		–	–
		APC-H7	+		–	–
		FITC	+	+	+	+/–
		PE	+	+	+	
		PE-Cy7	+		–	–
		PerCP	+/–	+/–	–	–
		PerCP-Cy5.5	+	+	+	
	2ST8.5H7	PE	+	–	–	–
Human CD11a/LFA-1	G43-25B	PE	+	+/–	–	
	Hi111	APC	+	–	–	
		PE	+	+/–	–	
Human CD11b	ICRF44	Alexa Fluor® 488	+	–	–	–
		APC	+	–	–	–
		APC-Cy7	–	–	–	–
		FITC	–	–	–	–
		PE	+	+/–	+/–	–
		PE-Cy5	+		–	–
		PE-Cy7	+	–	–	–
Human CD11c	BLy6	APC	+	+/–	+/–	
		PE	+	+/–	+/–	
Human CD13	WM-15	PE		+/–	+/–	
Human CD14	M5E2	Alexa Fluor® 488	+	+/–	–	+
		Alexa Fluor® 700				–
		APC	+	+/–	–	+
		FITC	+	+/–	–	–
		PE	+	+/–	–	+
		PE-Cy7	+	–	–	+
		PerCP-Cy5.5	+		–	+
	MOP9	PE	+	–	–	–
		APC	+	–	–	–
		PerCP	+	–	–	–
		APC-Cy7	+	–	–	–
Human CD15	W6D3	PE		+	+	
	HI98	PE		+	+	

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Specificity	**Clone**	**Fluorochrome**				
Human CD16	3G8	Alexa Fluor® 647			–	–
		Alexa Fluor® 700			–	–
		APC-Cy7			–	–
		FITC	+/–	+/–	–	–
		Pacific Blue™			–	–
		PE	+	+/–	–	–
		PE-Cy7	+	–	–	–
	B73.1	PE†	+	+	+	+
	NKP15	FITC		–	–	–
	G022	Purified	–	–	–	–
Human CD18	6.7	APC	+	+	+	
		PE	+	+	+	
Human CD19	HIB19	Alexa Fluor® 488	–			–
		Alexa Fluor® 700	–	–	–	–
		APC	+	+/–	–	
		FITC	+	+/–		–
		PE	+	+/–	–	–
		PE-Cy7	–	–	–	–
	SJ25C1	AmCyan	–		–	–
		APC	+/–	+/–	–	–
		APC-Cy7			–	–
		APC-H7	–	–	–	–
		FITC	–	–	–	–
		PE	+	+/–	+/–	+/–
		PE-Cy7	+/–	–	–	–
		PerCP	–		–	–
		PerCP-Cy5.5	–		–	–
	4G7	FITC	+/–		–	–
		PE	–	–	–	–
		PerCP	–	–	–	–
Human CD20	2H7	APC	+	–	–	–
		FITC	+/–	–	–	–
		PE	+	–	–	–
		PE-Cy5				–
	L27	APC	+			–
		APC-Cy7	–	–	–	–
		FITC	–	–	–	–
		PE	+	–	–	–
		PE-Cy7	+	–	–	–
		PerCP	–	–	–	–
		PerCP-Cy5.5	–	–	–	–
Human CD20 (I/C)	H1 (FB1)	Alexa Fluor® 488	+	+	+	+
		Alexa Fluor® 647	+	+	+	+
		PerCP-Cy5.5	+	+	+	+
Human CD23	M-L233	PE	+	+/–	–	
Human CD25	M-A251	Alexa Fluor® 647	+	+	+	+
		APC	+	+	–	–
		FITC	+	+	+	–
		PE	+	+	+	+
		PE-Cy7	+	+	–	–
	2A3	PE	+	+	+	+
		APC	+	+	+	+
		FITC	+	+	+	+
		PE-Cy7	+	–	–	–

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Specificity	**Clone**	**Fluorochrome**				
Human CD27	L128	APC	+	+	+	
		FITC	+	+	+	
		PE	+	+	+	+
	M-T271	PE	–	–	–	–
Human CD28	CD28.2	APC	–	–	–	–
		FITC		–	–	
		PE		–	–	
Human CD31	WM59	PE	+	+/–	+/–	
Human CD33	WM53	PE	+	+/–	+/–	
	p67.6	APC				+
		FITC				–
		PE	+	+	+	+
		PE-Cy7				–
		PerCP-Cy5.5				–
Human CD34	8G12	APC	+		+	+/–
		FITC	+		+	+/–
		PE	+	+	+	+
		PE-Cy7	+		+/–	–
		PerCP	+	+	+	–
		PerCP-Cy5.5	+	+	+	–
	581	APC	+	+/–	–	+/–
		FITC	+	+	+	+/–
		PE	+	+	+	+
	563	PE	+	+	+	+
Human CD38	HB7	FITC	–	–	–	
		PE	–	–	–	
	HIT2	APC	–			
		PE	–	–	–	
Human CD40	5C3	APC	–	–	–	
		FITC		–	–	
		PE	–	–	–	
Human CD44	515	PE		+	+	
	G44-26	APC	+	+	+	+
		PE		+	+	
Human CD45	2D1	AmCyan			+	+
		APC			+	+
		APC-Cy7	+		+	+
		APC-H7	+		+	+
		FITC			+	+
		PerCP			+	+
		PerCP-Cy5.5			+	+
	HI30	APC	+		+	+
		FITC	+		+	+
		BD Horizon V450	+		+	+
		PE	+	+	+	+
		PE-Cy5	+		+	
		PE-Cy7	+		+	+
Human CD45RA	5H9	FITC	+	+	+	+
		PE	+	+	+	
	L48	FITC	+		+	+
	HI100	APC	+	+	+	+
		FITC	+	+	+	+
		BD Horizon V450	+		+	+
		PE	+	+	+	+
		PE-Cy5	+		+	+

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Specificity	**Clone**	**Fluorochrome**				
Human CD45RO	UCHL1	APC	–		–	–
		FITC	+	+	+	+
		PE	+	+	+	+
		PE-Cy5	+		+	+
Human CD54	HA58	PE	+/–	+/–	+/–	
Human CD56	B159	Alexa Fluor® 488	–			
		Alexa Fluor® 647	–			
		APC	–			
		PE	–	–	–	–
		PE-Cy7	–	–		
	MY31	PE	+/–	+/–	–	–
	NCAM16.2	APC			–	–
		FITC			–	–
		PE	–	–	–	–
		PE-Cy7			–	–
Human CD62L	DREG56	PE	–	–	–	–
Human CD69	FN50	APC	–	–	–	–
		PE	+/–	–	–	–
Human CD79a (I/C)	HM47	APC	+	+	+	
		PE	+	+	+	
Human CD80	L307.4	PE		+	+	
Human CD83	HB15e	APC	+			
		PE	+	+/–	+/–	
Human CD86	2331	APC	+			
		PE	+	+	+	
	IT2.2	PE	+	+	+	
Human CD94	HP-3D9	APC	+	+	+	
		FITC	+	+	+	
		PE	+	+	+	
Human CD95	DX2	APC	–	–	–	–
		FITC	–	–	–	–
		PE	–	–	–	–
		PE-Cy5	–	–	–	–
Human CD117	YB5.B8	APC	–	–	–	–
		PE	+	+	+	+
		PerCP-Cy5.5	–	–	–	–
Human CD123	7G3	PerCP-Cy5.5	–		–	+
Human CD127	hIL-7R-M21	PE	–		–	–
Human CD138	Mi15	PE	+	+	+	
Human CD161	DX12	APC	+/–	–	–	–
		FITC	–	–	–	–
		PE	–	–	–	–
		PerCP-Cy5.5	–	–	–	–
Human CD195	2D7	APC		–	–	
		FITC	–	–	–	
		PE		–	–	
		PE-Cy7	+/–	–	–	
	3A9	APC		–	–	
		PE	–	–	–	
DC-SIGN	DCN46	PE	+	+	+	

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Specificity	**Clone**	**Fluorochrome**				
HLA-DR	G46-6	APC	+	+	+	
		FITC	+	+	+/–	
		PE	+	+	+	
		APC	+	+	+	
	Tu36	APC	+			
		PE	+	+/–	–	
	L243	APC				+
		APC-Cy7				+
		PE				+
		PerCP-Cy5.5				+
IgD	IA6-2	PE	–	–	–	
IgM	G20-127	PE	+	+/–	+/–	

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Antibodies to Mouse Cell-Surface Markers Tested for BD Phosflow Protocols

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Specificity	**Clone**	**Fluorochrome**				
Mouse CD3	145-2C11	Alexa Fluor® 488		+	–	–
		APC	+		–	+/–
		APC-Cy7			–	–
		PE	+	+	–	+
		PE-Cy5			–	+/–
		PerCP		+/–	–	–
		PerCP-Cy5.5		+/–	–	+/–
	500A2	Alexa Fluor® 700			–	+/–
		Pacific Blue™			–	+
		PE		+	+	
	17A2	Alexa Fluor® 647	+	+	+	+
		Alexa Fluor® 700			–	+/–
		APC			+	+
		FITC	+	+	+	+
		Pacific Blue™			–	+/–
		PE		+	+/–	+
		PE-Cy5			+/–	+
		PerCP-cy5.5			–	+
Mouse CD4	GK1.5	APC-Cy7			–	–
		FITC		+	+	–
		PE	+	+	+	–
	RM4-5	Alexa Fluor® 488		+	+	–
		Alexa Fluor® 647		+	+	–
		Alexa Fluor® 700			+	–
		APC			+	–
		FITC		+	–	–
		Pacific Blue™			+	–
		PE		+	+	–
		PE-Cy5			+	–
		PE-Cy7			+	–
		PerCP		+	–	–
		PerCP-Cy5.5		+	+	–
	H129.19	FITC			+	–
		PE			+/–	–
		PE-Cy5			–	–
Mouse CD8	53-6.7	Alexa Fluor® 488		+	+	–
		Alexa Fluor® 647		+	+	–
		APC	+		+	–
		APC-Cy7			–	–
		FITC		–	–	–
		Pacific Blue™			+	–
		PE	+	+	+	–
		PE-Cy5			–	–
		PerCP		+	–	–
		PerCP-Cy5.5		+	–	–
	H35-17.2	PE			–	–
	53-5.8	FITC			–	–
		PE			–	–

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Antibodies to Mouse Cell-Surface Markers Tested for BD Phosflow Protocols *(continued)*

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Specificity	**Clone**	**Fluorochrome**				
Mouse CD11b	M1/70	Alexa Fluor® 488		+	+	+
		Alexa Fluor® 647			+	+
		Alexa Fluor® 700			+	+
		APC			+	–
		FITC	+	+/–	+/–	+
		PE	+	+	+	+
		PE-Cy7			+	+
		PerCP-Cy5.5		+	+	+
Mouse CD11c	HL3	Alexa Fluor® 647			+	+
		APC			+	+
		FITC			+	+
		PE	+	+	+	+
		PE-Cy7			–	–
Mouse CD19	1D3	PE	–	–	–	
		PerCP-Cy5.5		–	–	
Mouse CD21/35	7G6	FITC		–	–	
		PE	+/–		–	
Mouse CD23	B3B4	FITC	+/–		–	
		PE	+	–	–	
Mouse CD24	M1/69	FITC	+	–	–	
		PE	+	–	–	
Mouse CD25	7D4	APC			–	–
		FITC		+	+	+
		PE			+	+
	3C7	FITC			–	–
		PE		+	+	
	PC61	APC			–	–
		APC-Cy7			–	–
		PE			–	–
		PE-Cy7			–	–
		PerCP-Cy5.5			–	–
Mouse CD27	LG.3A10	PE			+	+
Mouse CD28	37.51	PE		–	–	
Mouse CD43	S7	PE	+		+	
Mouse CD44	IM7	APC		+	+	
		FITC		+	+	
		PE		+	+	
Mouse CD45	30F11	PE		+	+	
		PerCP-Cy5.5	+		+	
	14.8	PE			+	+
Mouse CD45R/B220	RA3-6B2	Alexa Fluor® 488	+	+	+	+
		Alexa Fluor® 647	+	+	+	+
		Alexa Fluor® 700			+	–
		APC	+	+	+	+
		APC-Cy7		–	–	–
		FITC	+	+	+	+
		Pacific Blue™	+	+	+	+
		PE	+	+	+	+
		PE-Cy5	+	+	+	+
		PE-Cy7	+	+	+	+
		PE-Texas Red®	+	+	+	+
		PerCP	+	+	+	–
		PerCP-Cy5.5	+	+	+	+
Mouse CD49b	DX5	FITC		–	–	–
		PE			+	+

Notes:
Blank indicates not tested
+ Good resolution between positive and negative peaks- Recommended
– Poor resolution between positive and negative peaks - Not Recommeded
+/– Donor dependent. The positive and negative peaks are not separated well for some donors

Antibodies to Mouse Cell-Surface Markers Tested for BD Phosflow Protocols *(continued)*

			Protocol I *Detergent method*	Protocol II *Mild alcohol method*	Protocol III *Harsh alcohol method*	Protocol IV *Detergent method*
Fix buffer recommended for PBMCs or cell lines:			BD Cytofix™ Buffer (554655)	BD Cytofix™ Buffer (554655)	BD Cytofix™ Buffer (554655)	BD Cytofix™ Buffer (554655)
Fix buffer recommended for whole blood, splenocytes, bone marrows, and other sample types containing erythrocytes:			BD™ Phosflow Lyse/Fix Buffer (558049)	BD™ Phosflow Lyse/Fix Buffer (558049)	BD™ Phosflow Lyse/Fix Buffer (558049)	BD™ Phosflow Lyse/Fix Buffer (558049)
Perm Buffer:			BD™ Phosflow Perm/Wash Buffer I (557885)	BD™ Phosflow Perm Buffer II (558052)	BD™ Phosflow Perm Buffer III (558050)	BD™ Phosflow Perm Buffer IV (560746)
Specificity	**Clone**	**Fluorochrome**				
Mouse CD69	H1.2F3	FITC		–	–	
		PE		–	–	
Mouse GR-1	RB6-8C5	FITC		+	+	
		PE	+	+	+	
Mouse I-A/I-E	M5/114.15.2	PE		+	+	
	2G9	FITC		+	+	
Mouse IgD	11-26.c2a	FITC	+		–	
Mouse IgM	II/41	APC	+			
		FITC	+		–	
	R6-60.2	PerCP-Cy5.5	+		–	
Mouse Ly6C	AL-21	FITC	+	–	–	
Mouse I-A(b)	AF6-120.1	FITC	+/–	–	–	
		PE	+	–	–	
Mouse NK1.1	PK136	APC			–	+
		FITC	–	–	–	–
		PE	+	–	–	+
		PE-Cy7			–	–
		PerCP-Cy5.5			–	–
Mouse TCR	H57-597	APC	+	+	+	+
		FITC		+	+	–
		PE	+	+	+	+

Notes:
Blank indicates not tested
+ Good resolution between positive and negative peaks- Recommended
– Poor resolution between positive and negative peaks - Not Recommeded
+/– Donor dependent. The positive and negative peaks are not separated well for some donors

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APC-Cy7: US patent 5,714,386

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