

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 <i>Detergent method</i>	Protocol II <i>Mild alcohol method</i>	Protocol III <i>Harsh alcohol method</i>	Protocol IV <i>Detergent method</i>	
Fix buffer recommended for PBMCs or cell lines:			BD Cytotfix™ Buffer (554655)	BD Cytotfix™ Buffer (554655)	BD Cytotfix™ Buffer (554655)	BD Cytotfix™ 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	+	+	+/-	-	
	UCHT1	PerCP-Cy™5.5	+	+	+	+	
		Alexa Fluor® 488	+	+	+	+	
		Alexa Fluor® 647	+	+	+	+	
		Alexa Fluor® 700	+	+	+/-	-	
		APC	+	+	+	+	
		FITC	+	+	+	+	
		BD Horizon™ V450			+	+	
		Pacific Blue™		+	+	+	
		PE	+	+	+	+	
		PE-Cy™5	+	+	+	+	
		PE-Cy7	+	+	+	+	
		APC	+	-	-	+/-	
		HIT3a	FITC		-	-	-
			PE	+	-	-	-
PE-Cy5	+			-	-		
SP34	PE-Cy7	+		-	+/-		
	PerCP	+	+	+	-		
Human CD4	L200	FITC	+	+	+		
		PE	+	+	+	-	
		PE-Cy7	+	+	+	-	
		PerCP	+	+	+	-	
	RPA-T4	PerCP-Cy5.5	+	+	+		
		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	+		+	+	-		
L120	PerCP-Cy5.5	+	+	+	+		
	PE	-	-	-	-		
Human CD5	UCHT2	PE	+	+	+		
		APC	+	+/-	-		

Notes:

Blank indicates not tested

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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-Cy7	+	+	-	+
	SK1	APC	+	+	+	+
		APC-Cy7	+/-		-	-
		APC-H7	+		-	-
		FITC	+	+	+	+/-
		PE	+	+	+	
		PE-Cy7	+		-	-
PerCP		+/-	+/-	-	-	
2ST8.5H7	PerCP-Cy5.5	+	+	+		
	PE	+	-	-	-	
Human CD11a/LFA-1	G43-25B	PE	+	+/-	-	
	Hi111	APC	+	-	-	
		PE	+	+/-	-	
Human CD11b	ICRF44	Alexa Fluor® 488	+	-	-	-
		APC	+	-	-	-
		APC-Cy7	-	-	-	-
		FITC	-	-	-	-
		PE	+	+/-	+/-	-
		PE-Cy5	+		-	-
Human CD11c	Bly6	APC	+	+/-	+/-	
		PE	+	+/-	+/-	
Human CD13	WM-15	PE		+/-	+/-	
Human CD14	M5E2	Alexa Fluor® 488	+	+/-	-	+
		Alexa Fluor® 700				-
		APC	+	+/-	-	+
		FITC	+	+/-	-	-
		PE	+	+/-	-	+/-
		PE-Cy7	+	-	-	+
	MOP9	PerCP-Cy5.5	+		-	+
		PE	+	-	-	-
		APC	+	-	-	-
		PerCP	+	-	-	-
Human CD15	W6D3 HI98	APC-Cy7	+	-	-	-
		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*	+	+	+	+		
Human CD18	6.7	NKP15	FITC	-	-	-		
		G022	Purified	-	-	-	-	
Human CD19	H1B19	APC	+	+	+			
		PE	+	+	+			
		Alexa Fluor® 488	-			-		
		Alexa Fluor® 700	-	-	-	-		
		APC	+	+/-	-	-		
	SJ25C1	SJ25C1	FITC	+	+/-	-	-	
			PE	+	+/-	-	-	
			PE-Cy7	-	-	-	-	
			AmCyan	-		-	-	
			APC	+/-	+/-	-	-	
		4G7	4G7	APC-Cy7	-		-	-
				APC-H7	-	-	-	-
				FITC	-	-	-	-
				PE	+	+/-	+/-	+/-
				PE-Cy7	+/-	-	-	-
				PerCP	-	-	-	-
Human CD20	2H7	PerCP-Cy5.5	-		-	-		
		APC	+	-	-	-		
		FITC	+/-	-	-	-		
		PE	+	-	-	-		
	L27	L27	PE-Cy5					
			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	+	+	+	+		
	2A3	2A3	PE-Cy7	+	+	-	-	
			PE	+	+	+	+	
			APC	+	+	+	+	
			FITC	+	+	+	+	
			PE-Cy7	+	-	-	-	

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Specificity	Clone	Fluorochrome					
Human CD27	L128	APC	+	+	+		
		FITC	+	+	+		
		PE	+	+	+	+	
Human CD28	CD28.2	APC	-	-	-	-	
		FITC					
Human CD31	WM59	PE	+	+/-	+/-		
		WM53	PE	+	+/-	+/-	
Human CD33	p67.6	APC				+	
		FITC				-	
		PE	+	+	+	+	
		PE-Cy7				-	
		PerCP-Cy5.5				-	
Human CD34	8G12	APC	+		+	+/-	
		FITC	+		+	+/-	
		PE	+	+	+	+	
		PE-Cy7	+		+/-	-	
		PerCP	+	+	+	-	
	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			+	+	
	HI30	PerCP-Cy5.5			+	+	
		APC	+		+	+	
		FITC	+		+	+	
		BD Horizon V450	+		+	+	
Human CD45RA	5H9	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	-		-	-
	MY31	PE-Cy7	-		-	-
		PE	+/-	+/-	-	-
		APC			-	-
		FITC			-	-
NCAM16.2	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	+	+	+	
Human CD94	HP-3D9	APC	+	+	+	
		FITC	+	+	+	
		PE	+	+	+	
Human CD95	DX2	APC	-	-	-	
		FITC	-	-	-	
		PE	-	-	-	
		PE-Cy5	-	-	-	
Human CD117	YB5.88	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	+/-	-		-		
DC-SIGN	DCN46	APC		-	-	
		PE	+	+	+	

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

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

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Mouse CD11b	M1/70	Alexa Fluor® 488		+	+	+
		Alexa Fluor® 647			+	+
		Alexa Fluor® 700			+	+
		APC			+	-
		FITC	+	+/-	+/-	+
		PE	+	+	+	+
		PE-Cy7			+	+
Mouse CD11c	HL3	PerCP-Cy5.5		+	+	+
		Alexa Fluor® 647			+	+
		APC			+	+
		FITC			+	+
Mouse CD19	1D3	PE	+	+	+	+
		PE-Cy7			-	-
Mouse CD21/35	7G6	PE	-	-	-	-
		PerCP-Cy5.5			-	-
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	+		+	+
Mouse CD45R/B220	RA3-6B2	14.8			+	+
		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 Recommended

+/- 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 <i>Detergent method</i>	Protocol II <i>Mild alcohol method</i>	Protocol III <i>Harsh alcohol method</i>	Protocol IV <i>Detergent method</i>
Fix buffer recommended for PBMCs or cell lines:			BD Cytotfix™ Buffer (554655)	BD Cytotfix™ Buffer (554655)	BD Cytotfix™ Buffer (554655)	BD Cytotfix™ 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 2G9	PE		+	+	
		FITC		+	+	
Mouse IgD	11-26.c2a	FITC	+		–	
Mouse IgM	II/41 R6-60.2	APC	+			
		FITC	+		–	
		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 Recommended

+/- 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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