

## PBL 6 and 16 weeks whole blood

BD LSRII instrument configuration

Lasers	PMT detectors	Dichroic LP mirrors	BP filters	16 wk Panels 1&2	6 wk single panel
Blue 488nm 20mW	A	755	780/60	PE-Cy7	PE-Cy7
	B	685	695/40	PerCP-Cy5.5	PerCP-Cy5.5
	C	655	660/20		
	D	600	610/20	PE-CF594	PE-CF594
	E	550	575/26	PE	PE
	F	505	530/30	FITC	FITC
	G		488/10	SSC	SSC
	H				
Red 633nm 17mW	A	755	780/60	APC-H7	APC-H7
	B	710	730/45	Alexa 700	Alexa 700
	C		660/20	APC	APC
Violet 405nm 25mW	A	505	525/50	BV510	BV510
	B		450/50	BV421/V450	BV421
	C				
UV 355nm 20mW					

## Panel design

### PBL 16 weeks whole blood

#### T cell panel 1

Antibody and fluorochrome		Channel	Dilution (on 2x mix)	Dilution	Supplier	Cat. no	Clone
CD4	BV510	Amcyan	1500	3000	Biolegend	RM4-5	100553
CD8a	APC-H7	APC-Cy7	100	200	BD	53-6.7	560182
KLRG1	PE-Cy7	PE-Cy7	250	500	Biolegend	2F1	138416
CD161/NK1.1	BV421	Pacific Blue	300	600	Biolegend	PK136	108731
TCR $\alpha\beta$	PerCP-Cy5.5	PerCP-Cy5.5	300	600	Biolegend	H57-597	109228
TCR $\gamma\delta$	APC	APC	300	600	Biolegend	GL3	118116
CD62L	PE-CF594	PE-Texas Red	1000	2000	BD	MEL-14	562404
CD44	FITC	FITC	1000	2000	BD	IM7	553133
CD25	PE	PE	250	500	Biolegend	PC61	102008
CD45	Alexa 700	Alexa 700	300	600	Biolegend	30-F11	103128

#### B cell/Myeloid panel 2

Antibody and fluorochrome		Channel	Dilution (on 2x mix)	Dilution	Supplier	Clone	Cat. no
Ly6C	PerCP-Cy5.5	PerCP-Cy5.5	2500	5000	Biolegend	HK1.4	128012
CD11b	PE-Cy7	PE-Cy7	1000	2000	Biolegend	M1/70	101216
Ly6G	V450	Pacific Blue	300	600	BD	1A8	560603
IgD	BV510	AmCyan	1000	2000	BD	11-26c.2a	563110
CD115	APC	APC	250	500	Biolegend	AF598	135510
CD19	PE-CF594	PE-Texas Red	1000	2000	BD	ID3	562291
Ly6B	FITC	FITC	500	1000	AbD Serotec	7/4	MCA771FB
I-A/I-E	PE	PE	2000	4000	Biolegend	M5/114.15.2	107608
CD45	Alexa 700	Alexa 700	300	600	Biolegend	30-F11	103128

## PBL 6 weeks whole blood

T and B cell single panel

Antibody and fluorochrome		Channel	Dilution (on 2x mix)	Dilution	Supplier	Clone	Cat. no
CD4	BV510	Amcyan	1500	3000	Biolegend	RM4-5	100553
CD8a	APC-H7	APC-Cy7	100	200	BD	53-6.7	560182
CD19	PE-CF594	PE-Texas Red	1000	2000	BD	1D3	562291
CD161/NK1.1	BV421	Pacific Blue	300	600	Biolegend	PK136	108731
TCR $\alpha\beta$	PerCP-Cy5.5	PerCP-Cy5.5	300	600	Biolegend	H57-597	109228
TCR $\gamma\delta$	APC	APC	300	600	Biolegend	GL3	118116
CD62L	PE-Cy7	PE-Cy7	750	1500	Abcam*	MEL-14	ab25569
CD44	FITC	FITC	1000	2000	BD	IM7	553133
CD25	PE	PE	250	500	Biolegend	PC61	102008
CD45	Alexa 700	Alexa 700	300	600	Biolegend	30-F11	103128

\*Alternatively CD62L PE-Cy7 can be supplied by Biolegend