WHOLE BLOOD PANELS CMV/ageing – Laboratory Protocol (Brighton Immunology Lab)
Overview
Version 2, 03/10/2014

Detector (LSR II)	LP	BP	Fluorochrome	T cells	Treg	Th	TCR
Violet 1		450/50	PB	CD3	CD3	CD3	CD3
Violet 2	505	525/50	BV510	CD4	CD4	CD4	CD4
Violet 3	595	610/20	BV605	CCR7	CCR7	CCR7	CCR7
Red 1		660/20	APC	CD27	CD127	CCR6	TCR gd
Red 2	710	730/45	Alexa 700	CD45	CD45	CD45	CD45
Red 3	755	780/60	APC H7	CD8a	optional	CD8a	CD8a
Blue 1	505	530/30	FITC	CD45RA	CD45RA	CD45RA	CD45RA
Blue 2	550	575/26	PE	CD57	CD25	CCR10	Vdelta2
Blue 3			PETR/ECD etc.	optional	optional	optional	optional
Blue 4	635	670/14	PerCP(Cy5.5)	CD45RO	CD45RO	CCR4	CD16
Blue 5	755	780/60	PECy7	CD28	CD39	CXCR3	TCR ab

WHOLE BLOOD PANELS CMV/ageing – Laboratory Protocol (Brighton Immunology Lab)
antibodies in T cell panels
version 2, 03/10/2014

Fluorochrome	Specificity	Source	Clone	Order number
Pac Blue	CD3	Biolegend	UCHT1*	300418
BV510	CD4	Biolegend	OCKT4	317434
BV605	CCR7	Biolegend	G043H7	353223
APC	CD27	Biolegend	M-T271*	356409
APC	CD127	Biolegend	A019D5	351315
APC	CCR6	Biolegend	G034E3	353415
APC	TCR gd	Biolegend	B1	331211
AF700	CD45	Biolegend	HI30	304024
APC-H7	CD8a	BD	SK1	560179
FITC	CD45RA	Biolegend	HI100	304105
PE	CD57	Biolegend	HCD57	322311
PE	CD25	Biolegend	M-A251	356103
PE	CCR10	Biolegend	6588-5	341503
PE	Vd2	Biolegend	B6	331407
PCPCY5.5	CD45RO	Biolegend	UCHL1	304221
PCPCY5.5	CCR4	Biolegend	L291H4	359405
PCPCY5.5	CD16	Biolegend	3G8	302027
PECY7	CD28	Biolegend	CD28.2	302925
PECY7	CD39	Biolegend	A1	328211
PECY7	CXCR3	Biolegend	G025H7	353719
PECY7	TCR ab	Biolegend	IP26	306719

WHOLE BLOOD PANELS CMV/ageing – Laboratory Protocol (Brighton Immunology Lab)
Staining amounts required (after titration)
Version 2, 03/10/2014

T cell

Antibody	Test Volume (µl)
PB anti hCD3	1.00
BV510 anti hCD4	2.50
BV605 anti hCCR7	5.00
APC anti hCD27	0.63
A700 anti hCD45	1.00
APC/H7 anti hCD8a	1.25
FITC anti hCD45RA	1.25
PE anti hCD57	5.00
PerCP/Cy5.5 anti hCD45RO	2.50
PE/Cy7 anti hCD28	2.50
Total antibody volume	**22.63**
PBS Required	27.38

T reg

Antibody	Test Volume (µl)
PB anti hCD3	1.00
BV510 anti hCD4	2.50
BV605 anti hCCR7	5.00
APC anti hCD127	5.00
A700 anti hCD45	1.00
FITC anti hCD45RA	1.25
PE anti hCD25	1.25
PerCP/Cy5.5 anti hCD45RO	2.50
PE/Cy7 anti hCD39	0.63
Total antibody volume	**20.13**
PBS Required	29.88

T helper

Antibody	Test Volume (µl)
PB anti hCD3	1
BV510 anti hCD4	2.5
BV605 anti hCCR7	5
APC anti hCCR6	5
A700 anti hCD45	1
APC/H7 anti hCD8a	1.25
FITC anti hCD45RA	1.25
PE anti hCCR10	2.5
PerCP/Cy5.5 anti hCCR4	1.25
PE/Cy7 anti hCXCR3	2.5
Total antibody volume	**23.25**
PBS Required	26.75

TCR

Antibody	Test Volume (µl)
PB anti hCD3	1.00
BV510 anti hCD4	2.50
BV605 anti hCCR7	5.00
APC anti TCRγδ	5.00
A700 anti hCD45	1.00
APC/H7 anti hCD8a	1.25
FITC anti hCD45RA	1.25
PE anti hTCRVδ2	1.25
PerCP/Cy5.5 anti hCD16	2.50
PE/Cy7 anti hTCRαβ	5.00
Total antibody volume	**25.75**
PBS Required	24.25

Whole blood stain, lyse and wash procedure

1) Place required antibodies/aliquot of antibody master mix in Falcon 352052 tubes (see table below and panels);
2) Add PBS *ad* 50 µl (PBS can be added to master mix beforehand, in this case dispense 50 µl of antibody mastermix/PBS in each tube as a first step and continue with step 3);
3) Add 100 µl of whole blood (sodium heparin);
4) incubate 20 minutes at room temperature in the dark;
5) During incubation dilute RBC LYSIS/FIXATION solution in deionised water (1:10 dilution)
6) Add LYSIS/FIXATION solution to each tube and mix well (pipette)
7) Incubate for 15 minutes at room temperature in the dark;
8) Centrifuge at 350g for 5 min;
9) Carefully remove supernatant;
10) Resuspend cell pellet (in 2ml of PBS);
11) Centrifuge at 350g for 5 min;
12) Carefully remove supernatant;
13) RESUSPEND CELL PELLET (300 µl cell staining buffer);
14) Filter samples prior to acquisition (transfer to Falcon 352235 tubes with strainer);
15) ANALYSE CELLS IMMEDIATELY OR STORE AT 4°C in the dark.

If beads are used for absolute counting in a modified protocol it may be required to leave out the wash step.

Suggested QC for BD LSR II: As a routine CS&T setup is completed prior to every run. In addition 8-peak rainbow beads are acquired before sample acquisition (if you prefer, your software may allow you to link PMTs to CS&T runs). Rainbow beads are used to set PMTs to match the same target channels with respect to each peak and each fluorescence parameter every time the panel is run.

Note: not all parameters show 8 distinct peaks. Save a template document from your initial optimised run and replicate the target channels for all visible peaks each time (set gates on histograms). This ascertains comparability from time point to time point.