STAINING PROTOCOL FOR CYTOMETRY

Mix of Antibodies

Prepare a premix of antibodies in 1.5 ml Eppendorf tube according to dilutions required for each antibody.
Store premix at +4°C or on ice, protected from light until use.

For example 1

CD45	FITC
CD3	BV421
CD4	APC-H7
CD8	PECy7
CD19	APC
CD56	BV650

For exemple 2

CD45	FITC
CD3	PE
CD4	BV786
CD8	PECy7
CD19	APC
CD56	BV421

Ref. CYTO-COMP Cell Kit: cat. No 6607023 (Beckman Coulter France)
A material which is used to verify the performance of an assay intended to be used for the qualitative and/or quantitative detection of multiple cluster of differentiation (CD) cell markers in a clinical specimen, including associated percentage and/or enumeration of specific cell populations.

Single stained controls

1) Label 5 ml FACS tubes for each single staining
2) Add 1 drop of CYTO-COMP Cell Kit per 5 ml FACS tube
3) Add the required amount of antibody (1 antibody per tube)
4) Incubate in the dark at RT for 20min
5) Add 500µl 1xPBS
6) Keep at 4°C protected from light until acquisition

Cell Staining

1) Prepare one 5 ml FACS tube per sample (remember to include a tube for unstained control)
2) Transfer $1x10^6$ cells into the corresponding 5 mL tubes
3) Add 2 ml 1x PBS
4) Centrifuge 5 min at 1300 rpm, discard supernatant
5) Mix and spin shortly (about 20 seconds) antibody premix prior to using it
6) Add 50 µl of the premix into the corresponding tube
7) Mix shortly by vortexing
8) Incubate 20 min in the dark at 4°C
9) Add 2 ml 1x PBS
10) Centrifuge 5 min at 1300 rpm, discard supernatant
11) Resuspend in 500 µl 1x PBS
12) Acquire samples on cytometer