

MODE OPERATOIRE / OPERATING PROCEDURE	VERSION
<b>STAINING PROTOCOL FOR CYTOMETRY</b>	A

### 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  $1 \times 10^6$  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

