

Case Study for Flow FineTest® Cytometry Colour Matching

FCM Colour Matching and Human Detection Cases

Case 1: T Cell Detection in Human Blood

Target	Fluorescein	Clone ID	Cat.No
CD3	FITC	OKT-3	FITC-30004
CD4	PE	RPA-T4	PE-30005
CD8	APC	OKT-8	APC-30006

TIPS:

- ①Obvious grouping for CD3/CD4/CD8 without isotype control.
- ②Red blood cell lysis is very important. Excessive or insufficient lysis can lead to obscure grouping of lymphocyte.

Case 2: TH1 Cell Detection in Human Blood

Target	Fluorescein	Clone ID	Cat.No
CD3	FineTest®647	OKT-3	F647-30004
CD4	FITC	RPA-T4	FITC-30005
IFN-γ	PE	B27	PE-30053

TIPS:

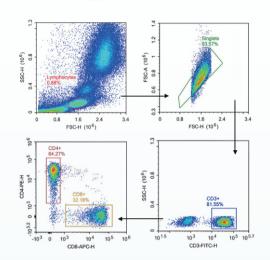
- $\ensuremath{\text{1}\text{-}\text{Obvious}}$ grouping for CD3/CD4 without isotype control. IFN- γ detection requires for isotype control.
- ②Stimulation of PMA can cause endocytosis of CD4+T cell epitope. CD4 antibody(Clone ID: RPA-T4) can reduce endocytic effects of CD4 epitope via detecting permeabilized CD4 epitope. Grouping of CD4 cell is clearer.
- $\ensuremath{\mathfrak{IFN-\gamma}}$ should be detected after blocking, fixation and permeabilization.

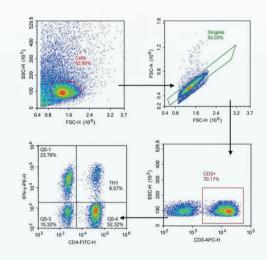
Case 3: Treg Cell Detection in Human Blood

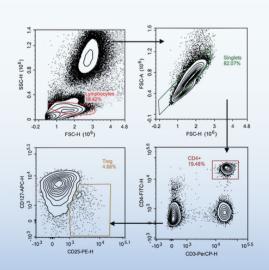
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Target	Fluorescein	Clone ID	Cat.No
CD3	PerCP/Cyanine5.5	OKT-3	PCP55-30004
CD4	FITC	RPA-T4	FITC-30005
CD25	PE	BC96	PE-30035
CD127	FineTest®647	A019D5	F647-30033

TIPS

- ①Detection steps for FOXP3 in human blood is complex. It's not easy to detect FOXP3 as well. Human Treg cell can be detected with CD127. The ratio of CD4+CD25+CD127-/low is equivalent to the ratio of Treg cell.
- ②Setting of single positive tube is required for regulating compensation.







FCM Colour Matching and Mouse Detection Cases

Case 1: T Cell Detection in Mouse Spleen

Target	Fluorescein	Clone ID	Cat.No
CD3	FITC	17A2	FITC-30127
CD4	PE	RM4-5	PE-30128
CD8	APC	53-6.7	APC-30003

TIPS:

- ①Obvious grouping for CD3/CD4/CD8 without isotype control.
- ②Red blood cell lysis is very important. Excessive or insufficient lysis can lead to obscure grouping of lymphocyte.

Case 2: TH1 Cell Detection in Mouse Spleen

Target	Fluorescein	Clone ID	Cat.No
CD3	FineTest®647	17A2	F647-30127
CD4	FITC	RM4-5	FITC-30128
IFN-γ	PE	XMG1.2	PE-30074

TIPS:

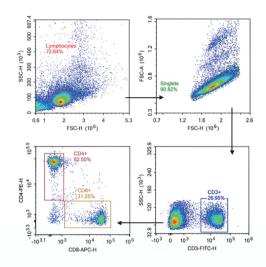
- ①Obvious grouping for CD3/CD4 without isotype control. IFN-γ detection requires for isotype control.
- ②Stimulation of PMA can cause endocytosis of CD4+T cell epitope. CD4 antibody(Clone ID: RM4-5) can reduce endocytic effects of CD4 epitope via detecting permeabilized CD4 epitope. Grouping of CD4 cell is clearer.
- ③As the cytokine, IFN-γ should be detected after blocking, fixation and permeabilization.

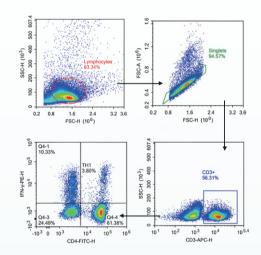
Case 3: Treg Cell Detection in Mouse Spleen

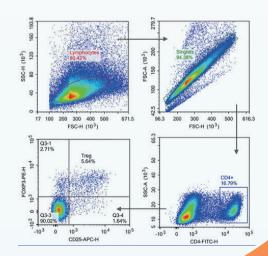
Target	Fluorescein	Clone ID	Cat.No
CD4	FITC	RM4-5	FITC-30128
CD25	APC	PC-61.5.3	APC-30017
FOXP3	PE	3G3	PE-30111

TIPS:

- ①Obvious grouping for CD4 without isotype control. Detection of CD25 and FOXP3 requires for isotype control.
- ②FOXP3 can't be detected using improper fixation and permeabilization kit. K081(Foxp3/Transcription Factor Staining Kit) is recommended.









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