

Rapid and easy intracellular + surface staining of MOUSE cells using PerFix-nc

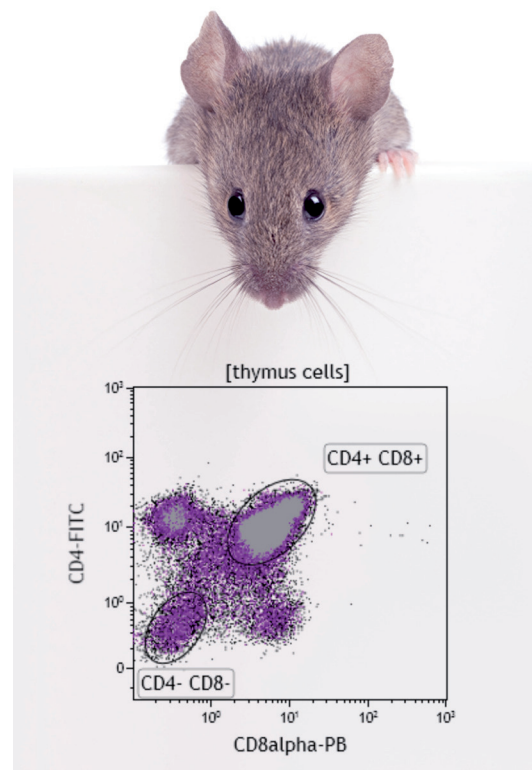
STAINING PROTOCOLS

Whole blood:

- Dispense 50 μL of cells into empty tube.
- Add 5 μL of fixative reagent R1 (low fixation), mix tube
- Incubate 15 min. at room temperature (RT).
- Add **300 μL** of permeabilizing reagent R2, and appropriate volume of antibodies (surface & intracellular), mix tube immediately.
- Incubate 15-30 min. at RT.
- Add 3 mL of final reagent 1X R3, mix tube.

Optional:

- Centrifuge 5 min. at 500 G, aspirate supernatant into waste.
- Re-suspend cells in 500 μL of final reagent 1X R3. Hold at 4°C, protect from light, until analysis.



TESTED REAGENTS

Specificity	Clone	Fluorochrome
CD3	145-2C11	PE
CD4	GK1.5	FITC
CD8	53-6-7	Pacific Blue*
CD11b	M1/70	APC
CD25	3C7	PE
CD44	IM7	APC
CD45R	30F11	PE
CD45RB	C363-16A	PE
CD62L	MEL-14	PE
CD122	TM β 1	PE
NK1.1	FJK-16	PE
TCR $\gamma\delta$	GL3	PE

Cells not in whole blood (spleen, thymus, sorted cells...):

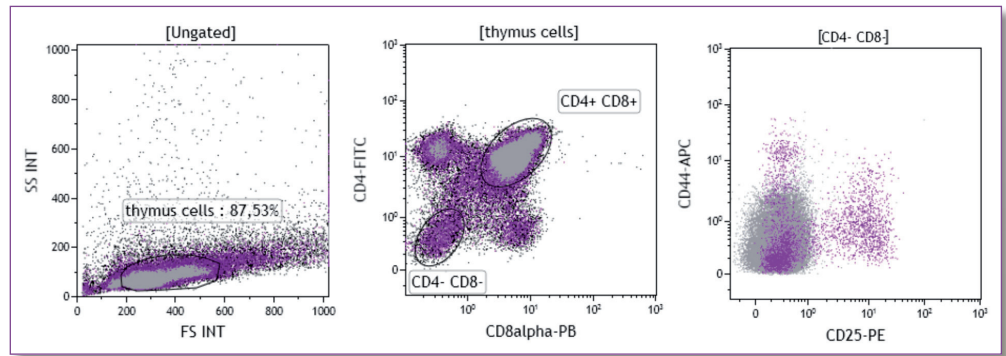
- Resuspend cells in 100% fetal calf serum (FCS) or equivalent.
- Dispense 50 μL of cells into empty tube.
- Add **2.5 μL** of fixative reagent R1 (low fixation), mix tube.
- Incubate 15 min. at RT.
- Add **150 μL** of permeabilizing reagent R2, and appropriate volume of antibodies (surface & intracellular), mix tube immediately.
- Incubate 15-30 min. at RT.
- Add **1.5 to 3 mL** of final reagent 1X R3, mix tube.

Optional:

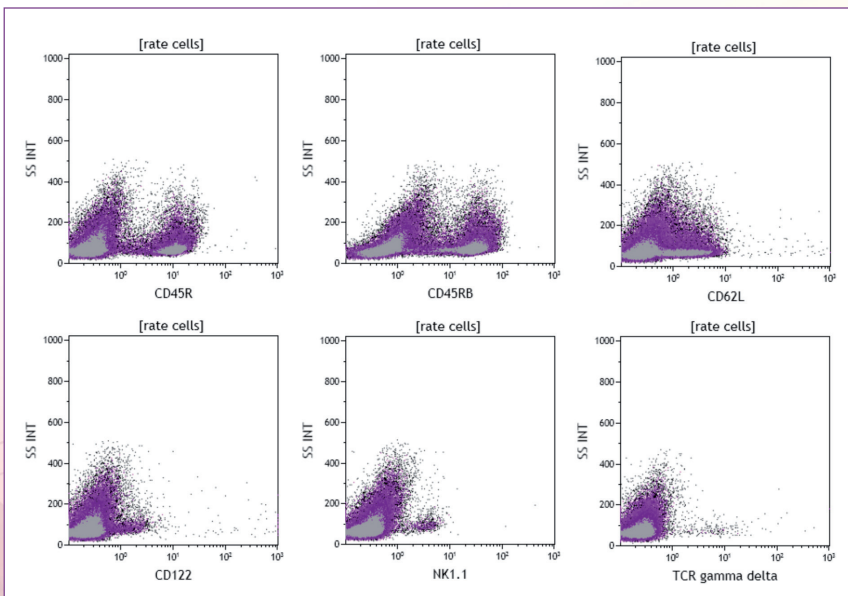
- Centrifuge 5 min. at 500 G, aspirate supernatant into waste.
- Re-suspend cells in 500 μL of final reagent 1X R3. Hold at 4°C, protect from light, until analysis.

RESULTS

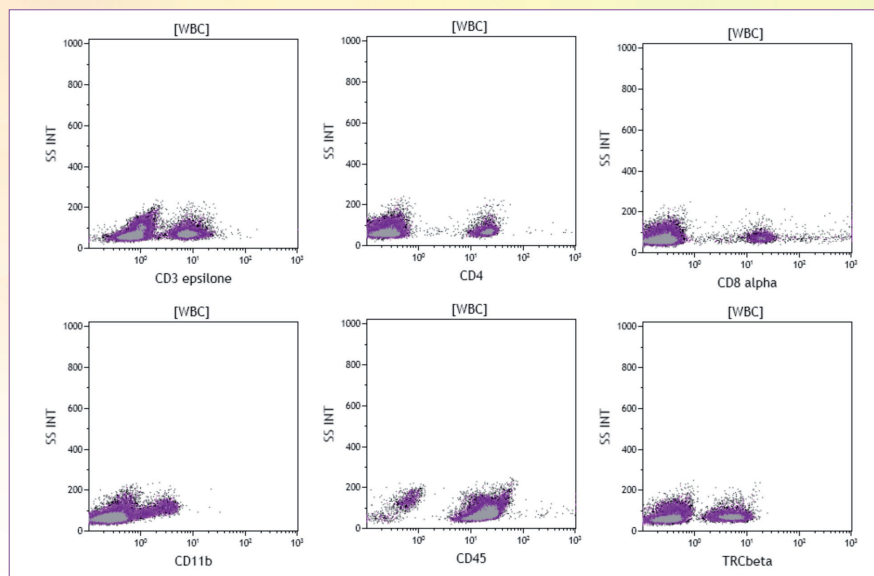
Mouse thymocytes analysis



Mouse splenocytes analysis



Mouse whole blood analysis



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