

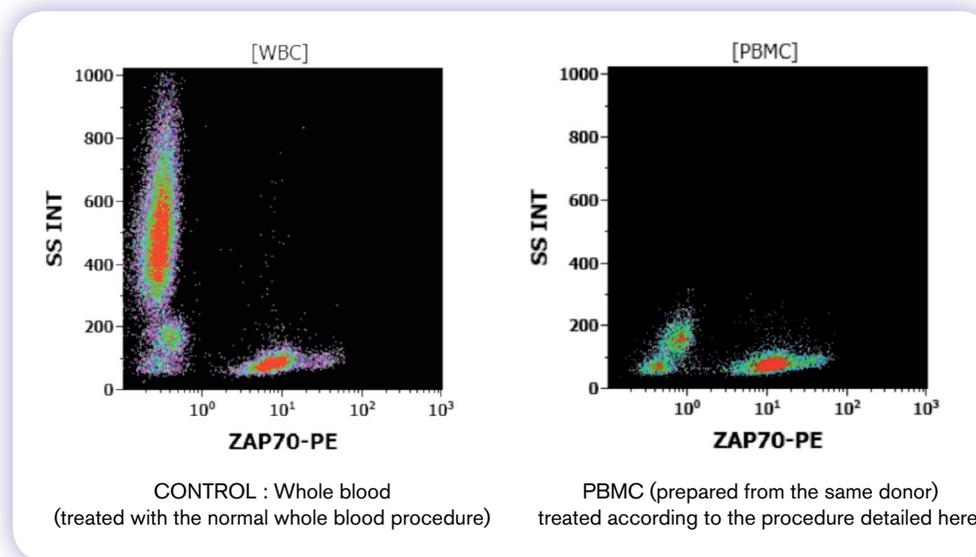


PerFix-nc, Procedure for PBMC (Peripheral Blood Mononuclear Cells) and purified cells

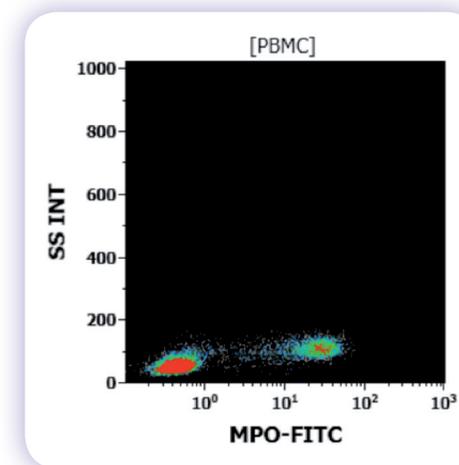
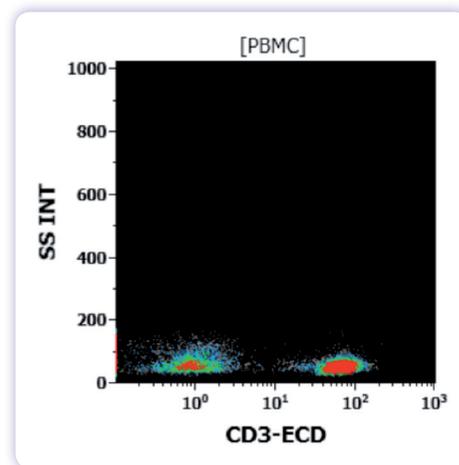
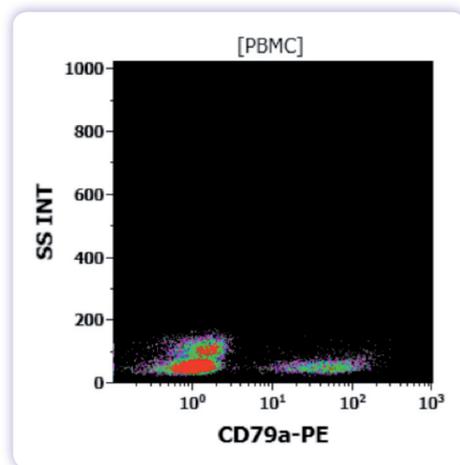
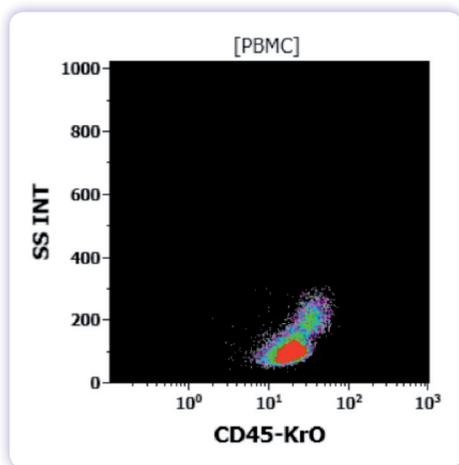
1. Resuspend the cells or PBMC in 100% serum (FCS or FBS), at a concentration ranging from «normal» ($5 \cdot 10^6/\text{mL}$) to «high» ($4 \cdot 10^7/\text{mL}$).
2. Pipet (as in the Blood procedure) 50 μL of these cells (e.g. 250,000 to 2 M cells per test), into the bottom of each appropriately labeled tube and add HALF the normal volume of R1: 2.5 μL .
3. Vortex immediately and incubate for 15 min. at room temperature (18 – 25°C).
4. Vortex the fixed cells and add HALF the normal volume of R2: 150 μL .
5. Immediately add the intracellular and gating markers (preferably, antibodies are pre-mixed with the Permeabilizing Reagent and added at the end of the fixation step).
6. Vortex immediately and incubate for 15-30 min. at room temperature (or 45 min. that is optimal for ZAP-70).
7. Add 1.5 mL to 3 mL of 1X R3 Reagent (prepared from the 10X concentrated R3 Solution); vortex immediately.
8. Optional: Centrifuge (500 g for 5 min.); resuspend in 500 μL of the 1X R3 Reagent

This procedure has been optimized for ZAP-70, MPO, CD79a, cCD3, and FoxP3 (with the same modification as for blood: 1 hour incubation + 1 extra PBS (3 mL) wash. See specific application note).

EXAMPLES OF RESULTS OBTAINED WITH THIS PROCEDURE



OTHER EXAMPLES: PBMC treated according to the procedure.



Tested products are labeled RUO (For Research use Only. Not for use in diagnostic procedures)

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