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» (5.10⁶/mL) to «high» (4.10⁷/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

CONTROL: Whole blood (treated with the normal whole blood procedure)

PBMC (prepared from the same donor) treated according to the procedure detailed here.

OTHER EXAMPLES: PBMC treated according to the procedure.