

## Direct Flow Cytometry Protocol

1. Transfer  $1-5 \times 10^6$  cells into a microtube. Centrifuge for 5' at 300 x g. Discard supernatant.
2. Carefully resuspend the cell pellet with 20-100  $\mu$ l of ice-cold Labeling Buffer (PBS + 2%BSA + 0.05%NaN<sub>3</sub>).
3. Add the staining antibody at the appropriate dilution. Incubate on ice protected from light for 30-60'.
4. Wash the unbound antibody by filling the microtube with Labeling Buffer, centrifuge for 5' at 300 x g and discard supernatant. Repeat washing step twice.
5. Resuspend the cells with ice-cold Labeling Buffer. Keep on ice protected from light until analyzed with a flow cytometer.