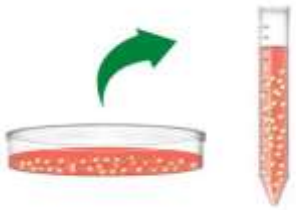
# Cell surface marker Direct Staining



3-6x$10^5$ cells / test

Detach cells from culture dish or flask

Wash cells with $PBS^-$ (1500 rpm for5 min) at 4°C, discard the supernatant

Add 500-1000 µl secondary host serum 10% (in lack of host serum, use BSA 5%)

Incubate for 20 min at RT or 30 min at 4°C

Wash cells with PBS- (1500 rpm for5 min) at 4°C, discard the supernatant

Split cells for Isotype control, unstain control and tests in separated tubes (each tube consist of 100 µl $PBS^-$)

Add primary Antibody (datasheet's recommendation), mix and incubate for 30-45 min at 4°C in dark

Add Isotype control (datasheet's recommendation), mix and incubate 30-45 min at 4°C in dark

Rinse the cells with 1ml $PBS^-$ (1500rpm for5 min)

Run and analysis on Flow Cytometer or fix samples by adding 100ul paraformaldehyde 1% and keep at 4°C in dark up to 24h