Indirect Intracellular Staining

6-9 x10^5 cells / test

Detach cells from culture dish or flask

Wash with PBS-(1500 rpm for 5min) at 4°C, discard supernatant

Add 100 µl cold paraformaldehyde 2-4%, incubate for 20 min at 4°C

Wash cells with PBS-/Tween-0/05% (1500 rpm for5 min), discard supernatant

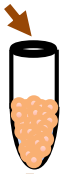
Permeabilization by adding 100 µl (0.1-0.2%)Triton X -100,incubate for 10min at RT

Wash cells with PBS-/Tween-0/05% (1500 rpm for5 min), discard supernatant

Add 800-1000 µl secondary host serum 10% (in lack of host serum, use BSA 5%) mix and incubate for 20 min at RT or 30 min at 4°C

Wash cells with PBS-/Tween-0/05% (1500 rpm for5 min)

Isolate cells pellet for Unstain control, Unstain control and tests in separated tubes (each tube consist of 100 µl perm/wash buffer



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

Rinse the cells with PBS-/Tween-0/05% (1500 rpm for5 min), discard supernatant

Add fluorescent dye conjugated secondary Antibody, mix and incubate for 30 min at RT or 45 min at 4°C in dark

Rinse the cells with PBS-/Tween-0/05% (1500 rpm for5 min) 2 times, remove the supernatant, and resuspend cells with 100 µl PBS-/Tween-0/05%

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