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Flow Cytometry

Materials

PBS:
8 g NaCl
0.2 g KCl
0.115 g $Na_2HPO_4 \times 2H_2O$
0.2 g KH_2PO_4
adjust pH to 7.4 and fill up to 1 liter with H O

FACS buffer:
PBS
5% fetal calf serum (FCS; heat-inactivated)
0.1% sodium azide (stock solution in water: 5%)

1st antibody:
tag-tools antibody
(starting dilution 1:200;
different dilutions should
be tested: 1:100 – 1:1,000)

2nd antibody:
secondary antibody
directed against 1st antibody,
fluorescence-labelled
(approx. dilution 1:500)

Useful controls:

- stain sample with irrelevant, isotype-matched primary antibody and secondary antibody
- stain sample with secondary antibody only
- stain cells not transfected with tagged construct with primary and secondary antibodies

Procedure

Preparation of cells:

1. aspirate medium and wash cells twice with 5-10 ml of PBS
2. detach cells with 1 ml trypsin/EDTA for max. 2 min
3. inactivate trypsin/EDTA with 4 ml serum-containing cell culture medium
4. transfer cell suspension into a 15 ml tube and pellet by centrifugation (3 min, 100g)
5. discard supernatant and resuspend the cell pellet in PBS and determine cell count
6. distribute into 1.5 ml reaction tubes (10^6 cells/tube)

Staining (perform all steps on ice):

1. pellet the cells by centrifugation (2 min, 2500 rpm), 4°C
2. resuspend cell pellet in 300 µl FACS buffer
3. add 1st antibody at desired concentration and incubate 1-1.5 h on ice
4. centrifuge 2 min, 2500 rpm, 4°C
5. discard supernatant and resuspend cell pellet in 600 µl FACS buffer
6. centrifuge again and wash 3 times with 600 µl FACS buffer
7. resuspend cell pellet in 300 µl FACS buffer
8. add fluorescently labelled 2nd antibody at desired concentration and incubate max. 30 min on ice (keep samples dark!)
9. wash twice with 600 µl FACS buffer
10. resuspend pellet in 600 µl FACS buffer and transfer to FACS tubes
11. analyse samples and controls by flow cytometry