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FLOW CYTOMETRY INTRACELLULAR & MEMBRANE STAINING PROTOCOL

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1a. Cell fixation (for membrane protein):

a. Suspend cells in 1x PBS buffer and wash them twice with 1x PBS buffer by centrifugation at 350-500 x g for 5 min each time. Discard the supernatant.
b. Re-suspend the cells in 1 ml of 1x PBS buffer briefly.
c. Fix the cells in a final concentration of 4% formaldehyde (or paraformaldehyde) for 20 min at room temperature.
d. Wash the cells 3 times with 1x PBS buffer by centrifugation at 350-500 x g for 5 min each time.

1b. Cell fixation and Permeabilization (for intracellular protein):

a. Permeabilize cells by adding 100% cold methanol slowly to pre-chilled cells to a final concentration of 90% methanol before incubating for 30 min on ice. Alternatively, fix the cells in a final concentration of 4% formaldehyde (or paraformaldehyde) for 20 min at room temperature. Then incubate the cells in 0.1% Triton X-100 in 1x PBS buffer for 15 min at room temperature.
b. Wash the cells 3 times with 1x PBS buffer by centrifugation at 350-500 x g for 5 min each time.

2. Immunostaining:

a. Blocking: Incubate the cells with 3 ml blocking buffer for 15-45 min at room temperature.
b. Add primary antibody at an appropriate dilution and incubate for 1 h at room temperature.
c. Wash the cells 3 times with 1x PBS buffer by centrifugation at 350-500 x g for 5 min each time.
d. Add diluted secondary antibody (enzyme or fluorescein conjugated or other types) to the cells and incubate for 45 min at room temperature.
e. Wash the cells 3 times with 1x PBS buffer by centrifugation at 350-500 x g for 5 min each time.
f. Re-suspend the cells in 0.5 ml 1x PBS buffer and analyze the results on a flow cytometer. For DNA staining, re-suspend the cells in 0.5 ml of DNA dye instead; incubate for at least 5 min at room temperature before analyzing the results on a flow cytometer.

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Buffers Needed

Blocking Buffer	1000 ml
Bovine serum albumin	5.00 g
1x PBS buffer	1000 ml

PBS Buffer	1000 ml
10 mM Na_2HPO_4	1.42 g
1.7 mM NaH_2PO_4	0.20 g
140 mM NaCl	8.19 g
Add ddH_2O to 1000 ml	
Adjust to pH 7.4	

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