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 1000 rpm 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 1000 rpm 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 1000 rpm for 5 min each time.

2. Immunostaining:
   a. Blocking: Incubate the cells with 3 ml blocking buffer for 1 h 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 1000 rpm for 5 min each time.
   d. Add diluted secondary antibody (enzyme or fluorescein conjugated or other types) to the cells and incubate for 1 h at room temperature.
   e. Wash the cells 3 times with 1x PBS buffer by centrifugation at 1000 rpm 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

<table>
<thead>
<tr>
<th>Buffer Type</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocking Buffer</td>
<td>1000 ml</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>5.00 g</td>
</tr>
<tr>
<td>1x PBS buffer</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PBS Buffer</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mM Na₂HPO₄</td>
<td>1.42 g</td>
</tr>
<tr>
<td>1.7 mM NaH₂PO₄</td>
<td>0.20 g</td>
</tr>
<tr>
<td>140 mM NaCl</td>
<td>8.19 g</td>
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</tbody>
</table>

Add ddH₂O to 1000 ml
Adjust to pH 7.4