

Flow cytometry (FC) Protocol

-Instruction

Flow cytometry is a technology to perform rapid quantitation and multiparameter analysis of single cells or other biological particles. It can analyze tensthousands of cells at high speed, and can simultaneously measure multiple parameters from one cell. When the special antibody labeled by fluorescence recognized the target antigen, the fluorescence signal will be acquire by Flow cytometry.

-Solution and reagents

4% formaldehyde

1%TritonX-100

10% normal goat serum

Primary antibody

Secondary-fluorescence antibody

-Sample preparation

Preparation for sample of cell

- 1. To suspension cell, collect cells by centrifugation at 1000rmp. To adherent cell, collect cells by trypsin digestion and collect by centrifugation at 1000rmp. The recommend number of cells are 1×10⁶.
- 2. Wash the cells by ice PBS for three times.

Preparation for sample of tissue

- 1. Sheared tissue and digestive tissue mass by pancreatin or collagenase.
- 2. Observe under microscope to single dispersed cell and terminate digestion.

Pancreatin often uses to digest less mesenchymal tissue, while collagenase works well for collagen structure. Generally, Pancreatin works well for 20-60min. While collagenase treats need 4-48h.

-Fixation and permeabilization

The fixation and permeabilization of samples are key steps that determine the success of your experiments. Cross-linking reagent (such as 4% formaldehyde, 10% formalin and glutaraldehyde) and denaturing reagent (such as 70% ethanol and 90% ethanol) are often used as a fixative. Cross-linking reagent works by crosslink with cellular proteins, while denaturing reagent can react with membrane and break membrane. So if we perform fixation by denaturing reagent, permeabilization is no need to label intracellular protein. But permeabilization must be performed after fixation by cross-linking reagent by



0.2% TritonX-100 to label intracellular protein. If the target protein is a membrane protein, permeabilization is needn't.

- 70% ethanol fix for 12h at 4℃ (It can keep at -20℃ for a month) or 4% formaldehyde fix for 15min at RT and permeabilization by 0.2% TritonX-100 for 5min(It can keep at 4℃ for one week). If the target protein is located on membrane, the fixtion can be ignored. To intracellular proteins, fixtion is needed.
- 2. Centrifuga at 1000rmop and wash cells by PBS for three times.

-Blocking

This process is to reduce the non-specific binding between antibodies and other proteins which is not specific to antibody. The blocking solution that we often use is the 10% normal goat serum (the species must be consistent with the secondary antibody).

-Antibody incubation

- 1. Prepare primary antibody solution by referring to the recommend dilution ratio.
- Incubate primary antibody at 4[°]C overnight. We need set a negative control at same time by adding normal rabbit or mouse IgG.
- 3. Wash cells with PBS for three times.
- 4. Prepare secondary antibody solution by referring to the recommend dilution ratio in dark.
- 5. Incubate secondary antibody at 4° C for 30min.
- 6. Wash cells with PBS for three times.
- 7. Re-suspend by 500µl PBS for detect.

-Detection

Keep the sample in dark and detect the fluorescence signal as soon as possible by a Flow cytometry.