

Preparation of Cells for Flow Cytometry

FC1

For the preparation of single cells derived from tissue culture cell lines.

Single cells must be suspended at a density of 10^5 - 10^7 cells/ml to keep the narrow bores of the flow cytometer and its tubing from clogging up. The concentration also influences the rate of flow sorting, which typically progresses at 2,000-20,000 cells/second. Phosphate Buffered Saline (PBS) is a common suspension buffer.

The most straightforward samples for flow cytometry are non-adherent cells from tissue cell culture. Here we describe methods for both tissue culture cell lines and adherent tissue culture cell lines. However analysis may be required from cells derived from other sources and Bio-Rad has a protocol (FC3) for the preparation of tissue from a variety of sources including; peritoneal macrophages, thymus cells, spleen cells and bone marrow. Further to this a protocol (FC2) is also available for the sample preparation of human peripheral blood mononuclear cells.

Reagents:

- Phosphate buffered saline (BUF036A) containing 1% bovine serum albumin (PBS/BSA)
- PBS
- 1x Accutase solution
- Trypsin 0.25%

Preparation of cells stored in liquid nitrogen

1. Prepare PBS/BSA.
2. Carefully remove cells from liquid nitrogen storage.
3. Thaw cells rapidly in a 37°C water bath.
4. Resuspend cells in cold PBS/BSA buffer and transfer them to a 15 ml conical centrifuge tube.
5. Centrifuge at 300-400 g for 5 min at 4°C.
6. Discard supernatant and resuspend pellet in an appropriate amount of cold (4°C) PBS/BSA, such as 10^7 cells/ml. **Note:** higher viability can be obtained by allowing the cells to recover in culture media overnight.

Preparation of tissue culture cell lines in suspension

This method provides a general procedure for use with tissue culture cells in suspension.

1. Prepare PBS/BSA.
2. Decant cells from tissue culture flask into 15 ml conical centrifuge tube(s).
3. Centrifuge at 300-400 g for 5 minutes at room temperature.
4. Discard supernatant and resuspend pellet in 10 ml of room temperature PBS/BSA.
5. Centrifuge at 300-400 g for 5 minutes at room temperature.

6. Discard supernatant and resuspend to a minimum concentration of 1×10^7 cells/ml in cold (4°C) PBS/BSA.

Preparation of adherent tissue culture cell lines

This method provides a general procedure for use with adherent tissue culture cells.

1. Prepare PBS/BSA.
2. Harvest cells by enzymatic release using 1x Accutase solution or 0.25% trypsin, followed by quenching with media containing serum. (**Note:** epitopes may be cleaved when using the enzymatic digestion method. Cells can also be harvested by gently scraping them into culture media)
 - I. Remove the culture medium and eliminate residual serum by rinsing cell monolayers with sterile, room temperature PBS.
 - II. Slowly add 1x Accutase solution or 0.25% Trypsin to cover the cell monolayer.
 - III. Incubate at 37°C for up to 10 minutes.
 - IV. After incubation gently tap the flask and the cells will detach and slide off in one sheet to the bottom of the flask.
 - V. Add growth medium and resuspend the cells by gently pipetting.
3. Centrifuge at 300-400 g for 5 min.
4. Discard supernatant and resuspend pellet in fresh, room temperature PBS/BSA to wash off any remaining cell debris and proteins.
5. Centrifuge at 300-400 g for 5 minutes at room temperature.

6. Discard supernatant and resuspend pellet in an appropriate amount of room temperature PBS/BSA.
7. Count cells using a hemocytometer or an automated cell counter such as the TC20™ Automated Cell counter (1450102 available from Bio-Rad.com).
8. Once counted dilute the cells with cold (4°C) PBS/BSA to a minimum concentration of 1×10^7 cells/ml.

For more information on flow cytometry resources visit bio-rad-antibodies.com/applications

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