Preparation of Peritoneal Macrophages, Bone Marrow, Thymus and Spleen Cells

Protocol

FC3

This method provides a general procedure for use with cell suspension cells acquired from the peritoneum, bone marrow, thymus and spleen. A certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques; these are guidelines only and may need to be adjusted for particular applications.

Reagents:

- Phosphate buffered saline (BUF036A) containing 1% bovine serum albumin (PBS/BSA)
- Ammonium chloride lysis buffer: 0.16M ammonium chloride, 0.17M Tris, pH 7.2
- Optional PBS/BSA with 25 µg/ml DNAse I or 5 mM EDTA to reduce cell aggregates

Method:

1. Prepare a single cell suspension from relevant tissue. Keep cells on ice to minimize cell death, which can lead to cell aggregation. Addition of DNAse I or EDTA can also reduce aggregation. Large aggregates can be removed by passing through a 40 µm cell strainer.
2. Centrifuge at 300-400 g for 5 minutes at 4°C.
3. Discard supernatant and resuspend pellet in 10 ml ammonium chloride lysis buffer.
4. Mix and incubate for 2 minutes at 4°C. **Do not exceed this time.**
5. Centrifuge at 300-400 g for 5 minutes at 4°C.
6. Discard supernatant and resuspend pellet in 10 ml cold (4°C) PBS/BSA
7. Centrifuge at 300-400 g for 5 minutes at 4°C.
8. Discard supernatant and resuspend pellet to a final volume of 10 ml with cold (4°C) PBS/BSA.
9. Count cells using a hemocytometer or an automated cell counter such as the TC20™ Automated Cell Counter (Bio-Rad product code 1450102).
10. Adjust suspension if necessary to give a final count of 0.7 – 1.2 x 10^7/ml.

Notes:

- The following should be considered when designing your flow cytometry experiments:
  - To avoid unspecific binding, you also need to block Fc receptors on cell types such as spleen cells, with FcR blocking reagents e.g. Bio-Rad's Mouse Seroblock reagent (BUF041).
  - Appropriate controls should always be carried out, for flow cytometry the following should be considered for inclusion;
    - Isotype controls used to determine if the staining is specific
    - Unstained cells should always be included in the experimental set-up to monitor autofluorescence.
- For all multi-color flow cytometry experiments it is advisable to include compensation controls and Fluorescence Minus One (FMO) controls, which assist with identifying gating boundaries.

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

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