Preparation of Human Peripheral Blood Mononuclear Cells

Protocol

FC2

This method provides a general procedure for use with peripheral blood mononuclear cells. 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 (PBS) (BUF036A) containing 1% bovine serum albumin (PBS/BSA)
- Histopaque or Ficoll

Method:

1. Allow separation media such as Histopaque to equilibrate to room temperature.
2. Dilute blood in equal volumes of room temperature PBS/BSA (for example, add 3 ml of PBS/BSA to 3 ml of blood).
3. Carefully overlay whole blood onto an equal volume of separation media in a 15 ml conical centrifuge tube.
4. Centrifuge at 300-400g for 30 minutes in a 20°C temperature controlled centrifuge with no brake. **Note:** Centrifugation at 4°C or with brake reduces efficiency of cell recovery.
5. Harvest cells from the serum/separation media interface using a pipet.
6. Place harvested cells in a 15 ml conical centrifuge tube.
7. Adjust the volume to 10 ml with PBS/BSA.
8. Centrifuge at 300-400 g for 5 minutes at room temperature.
9. Discard supernatant and resuspend pellet to a final concentration of at least 1 x 10^7 cells/ml with cold (4°C) PBS/BSA.

Notes:

- It is recommended that all containers which have come into contact with human blood or cells should be considered hazardous waste and discarded appropriately.
- 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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