Methods | Isolation of Human T Cell Populations by Panning

Method provided by Claudia Benike and Edgar G. Engleman, M.D., Stanford University School of Medicine, Department of Pathology.

This method is used to positively select specific lymphocyte subpopulations based on their cell surface markers. Total peripheral blood mononuclear cells or E-rosette positive (T) cells are incubated with monoclonal antibody, then poured into petri plates precoated with anti-mouse immunoglobulin. Cells positive (adherent) and negative (nonadherent) for the specific monoclonal antibody are collected after incubation.

Reagents:

1a. Peripheral blood mononuclear cells which have undergone a plastic adherence for monocyte depletion (1 hour at 37°C in RPMI 1640 with 5% human or fetal calf serum). Cells are decanted following incubation.

or

1b. Sheep erythrocyte (E) rosetted cells of human peripheral blood mononuclear cells (<3% surface immunoglobulin positive cells, <3% alphanaphthyl acetate positive cells). Centrifuge cells just prior to use, then remove supernatant.

2. Monoclonal antibody to human T lymphocyte subpopulation (e.g., anti-Leu-2a for T cytotoxic/suppressor cells; anti-Leu-3a for T helper/inducer cells)

3. Diluent: Fetal Calf Serum/Phosphate-Buffered Saline (FCS/PBS)

   a. Dulbecco's Phosphate-Buffered Saline (PBS) with calcium and magnesium

   b. 5% FCS/PBS
      5 ml fetal calf serum/100 ml PBS

   c. 1% FCS/PBS
      1 ml fetal calf serum/100 ml PBS

4. Goat anti-mouse immunoglobulin G (IgG), affinity purified

5. 0.05M Tris Buffer, pH 9.5

Equipment:

1. Plastic petri dishes, bacteriological grade (not treated for tissue culture), 15 x 100 mm
2. Refrigerator (2° - 8° C)

Procedure:

1. Dilute goat anti-mouse IgG to a concentration of 10 μg/ml in 0.05 M Tris.
2. Incubate each petri dish with 10 ml of diluted goat anti-mouse IgG at room temperature for 40 minutes. Remove unbound antibody by washing dish three times with PBS and one time with 1% FCS/PBS.
3. Dilute monoclonal antibody to 10 μg/ml in PBS.
4. Mix cell pellet (2 - 3 x 10^7 cells) with 20 μg (2 ml) of monoclonal antibody. Incubate at room temperature for 20 minutes. Centrifuge cells, remove supernatant, then wash with 5% FCS/PBS. Repeat centrifugation and washing. Resuspend ≤ 2 - 3 x 10^7 cells in 3 ml of 5% FCS/PBS and pour antibody-treated cells onto one coated plate. Incubate at 2° - 8° C for 2 hours.
5. Collect nonadherent cells by decanting. Wash plate gently with 5 ~~7~~ ml of 1% FCS/PBS. Decant. Repeat gentle washing four times, pooling washes. (Negative population)
6. Add 15 - 20 ml of 1% FCS/PBS to plate. Pipet vigorously to remove bound cells (positive population). Check plate for cells using an inverted microscope. If necessary, wash plate again with 1% FCS/PBS.

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