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Separation of T and B Lymphocytes with Magnetic Beads

Introduction

Immunomagnetic beads can be used in either a direct or indirect technique to isolate T and B cells. The direct method described below involves the use of magnetic beads coated with a monomorphic monoclonal antibody (CD2). CD2 is a determinant present on the cell surface of T lymphocytes and natural killer (NK) cells. Peripheral blood lymphocytes are obtained using a ficoll-hypaque method and are depleted of remaining granulocytes and monocytes by the use of iron filings. The cell suspension is then mixed with antibody coated magnetic beads which form rosettes around the CD2+ cells. In the indirect method a mouse anti-human monoclonal IgG antibody directed against CD2 is mixed with the lymphocyte suspension and binds to the cells bearing the specific antigen (CD2). The magnetic beads, in this case coated with sheep anti-mouse antibody, then form rosettes around each CD2+ cell. In either method, the CD2+ cells are isolated with the use of a magnet leaving a B cell enriched suspension. This suspension can then be used for DR typing in the Amos Modified (one wash) cytotoxicity technique using reduced incubation times. It is also possible to use an EDTA-pooled human serum elution medium to elute CD2+ cells from the magnetic beads. These eluted T cells can be used for HLA-ABC typing in the Amos Modified (one wash) technique.

HIFCS: heat-inactivated fetal calf serum
qs: quantity sufficient
LSR: Technicon lymphocyte separator reagent



Availability Codes	
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Reagents

Magnetic Beads

Direct: Dynal DynabeadsTM M-450; anti-CD2 antibody coated (Pan-T)
Concentration: about 4×10^8 beads/ml

Indirect: Dynal DynabeadsTM M-450; sheep anti-mouse or goat anti-mouse IgG antibody coated
Concentration: about 4×10^8 beads/ml

NOTE: Dynal DynabeadsTM are available from DYNAL, Inc., 45 North Station Plaza, Great Neck, NY 11021, U.S.A. Beads from other sources may be used for the indirect technique. We have experimented with beads from Advanced Magnetics, Inc. (Advanced Magnetics, Inc., 61 Mooney Street, Cambridge, MA 02138) and preliminary results look good. Due to the difference in the size of these beads, the technique requires some modifications.

Elution Medium

4 parts PHS
4 parts 2% EDTA
2 parts RPMI

Prepare fresh daily.

Procedure: Direct Method

1. Isolate lymphocytes by any method from peripheral whole blood, lymph node or spleen. It should be noted that this procedure works well when using frozen PBLs thawed by standard techniques. Use a total of $5 - 20 \times 10^6$ cells.
2. Deplete monocytes using carbonyl iron technique (Johnson, 1981) as follows:
NOTE: Residual granulocytes are also removed in this step.
 - a. Centrifuge the isolated lymphocytes for 10 min at 250g and resuspend the pellet in 2 ml HIFCS.
 - b. Transfer the suspension to a 15 ml round bottom polystyrene tube.
 - c. Add 6 ml Technicon LSR (Technicon Inst. Corp., 600 Hadley Rd., S. Plainfield, NJ 07080) and incubate at 37°C for 30 min with gentle rocking (a hematology type rocker is suggested).
 - d. After incubation, collect the carbonyl iron with a strong magnet. (Recommended: rare earth cobalt samarium magnet.)
 - e. Transfer the supernatant to another 15 ml round bottom tube and centrifuge for 10 min at 250g.
 - f. Resuspend the pellet in 2 ml 2% HIFCS-PBS; perform cell count and assess viability.
3. Using the stock solution of beads from Dynal, pipette beads into a five ml capped tube (12x75mm).

Use 100 μ l for $5-10 \times 10^6$ cells or 150 μ l for $10-20 \times 10^6$ cells.

4. Wash the beads 2X with PBS by filling the tube, capping it, and inverting to mix. Then, collect the beads with the magnet and discard the supernatant.
5. Add cell suspension to the beads and mix gently for 5 min at RT by gentle tilting and rotating. Do not use end-to-end inversion as this may result in mechanical damage to the cells.
6. Add 2 ml 2% HIFCS-PBS and isolate the cell-bead rosettes with the magnet. Allow 2-3 min for isolation.
7. Transfer the B cell-enriched supernatant into a 15 ml round bottom tube, add about 5 ml of 10% HIFCS-RPMI, mix and centrifuge for 10 min at 250g.
8. Resuspend the pellet in 0.5 ml RPMI; perform cell count and assess viability.

Procedure: Indirect Method

1. Harvest, purify and titrate a monoclonal antibody directed against CD2 from mouse ascites. Determine the working dilution and freeze aliquots at -80°C .
2. Centrifuge the cells obtained in steps 1 and 2a-e of the direct method for 10 min at 250g.
3. Resuspend the pellet in 5 ml of monoclonal antibody at working dilution.
4. Transfer the cell suspension to a 5-6 ml 12 x 75 mm polystyrene tube, cap the tube and mix gently for 5 min at RT by gentle tilting and rotating.
5. Centrifuge the cell suspension for 10 min at 250g, decant and blot.
6. Resuspend the cells in 5 ml 2% HIFCS-PBS and set aside.
7. Prepare magnetic beads as follows:
 - a. Pipette 1.0 ml magnetic beads coated with either goat anti-mouse IgG or sheep anti-mouse IgG into a 15 ml round bottom polystyrene tube.
 - b. Wash beads 2X with 6-8 ml PBS by adding the PBS to the tube, capping it, inverting it, collecting the beads with the magnet, and discarding the supernatant.
8. After last wash, add cell suspension (from step 6) to the beads and mix gently for 5 min at RT by gentle tilting and rotating. Do not use end-to-end inversion.
9. Collect the beads with magnet. Allow 2-3 min for isolation.
10. Transfer the B cell enriched supernatant into a 15 ml round bottom tube, add about 5 ml 10% HIFCS-RPMI, mix and centrifuge for 10 min at 250g.
11. Resuspend the pellet in 0.5 ml RPMI; perform cell count and assess viability.

Procedure: Elution of T Cells (Indirect Technique)

If using negative selection for B cells, T cells can be eluted from the beads by incubation with a FRESHLY prepared elution mixture.

1. Incubate coated beads with 5 ml elution media for 30 min at 37°C. Mix every ten minutes.
2. Collect the beads with a magnet; allow 2-3 min for isolation.
3. Transfer the supernatant (T cells) into a 15 ml round bottom tube and centrifuge for 10 min at 250g.
4. Resuspend the pellet in 1 ml RPMI; perform cell count and assess viability.

Troubleshooting

Weak or missing reactions with DQw specificities indicate monocyte contamination. Repeat the carbonyl iron technique. The viability of the separated cells is dependent upon the viability of the cells at the start of the procedure. If the starting viability is less than 80%, dead cells should be removed before starting the procedure or after separation.

If the cell yield is sufficient, the "ficoll" procedure is helpful in removing stray beads and red blood cells as well as improving the viability of the prep. Red cells can also be eliminated by use of a lysing buffer.

Results

The B cell yields average 12% of total cells added to the magnetic beads. Highly enriched (90-95%) populations of B cells and T cells with >95% viability are routinely obtained with these methods. Cells isolated in this way are ready for HLA typing using standard microlymphocytotoxicity methods. Since cells isolated by both the direct and indirect methods show increased reactivity, particular attention should be paid to incubation times and strength of complement. In general, incubation times should be decreased (suggested: cells + sera = 30 min; complement = 45 min) and the dilution of complement increased.

Common Variations

A rapid method for isolating T and/or B cells from peripheral whole blood using positive selection is available from DYNAL (Distributor, USA, Robbins Scientific Corporation).

References

Johnson A: Enrichment of B lymphocytes using an anti-human F(ab')₂ monolayer. In: AACHT Laboratory Manual; AA Zachary and WE Braun, eds.; AACHT, New York; pp. 1-9-1 to 1-9-8, 1981.

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