Enrichment of Cells of Interest from Heterogeneous Murine Cells with BioMag Goat Anti-rat IgG

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[Abstract] BioMag Goat Anti-Rat IgG is a standard BioMag particle coated with polyclonal goat anti-rat IgG antibodies and is highly suited for use in cell sorting methods where a rat IgG antibody is used as a primary antibody. BioMag Goat Anti-Rat IgG can be used to separate the cells of interest from a heterogeneous cell population using negative selection. BioMag Goat Anti-Rat IgG can also be used as a secondary antibody in enzyme immunoassays and radioassays that utilize a rat IgG primary monoclonal antibody.

The efficiency of enrichment by negative selection depends on antigen availability and the total cell population. The author has intensive experience about enrichment of mouse CD4CD8 double negative (DN) thymocytes from total thymocytes which contains only about 2% DN population. Usually the DN population occupies more than 80% of total thymocyte after one round of enrichment. However the depletion efficiency is much lower for bone marrow cells.

Materials and Reagents

1. Mouse total thymocytes
2. BioMag goat anti-rat IgG (QIAGEN, 50 ml, catalog number: 310104) (QIAGEN, 500 ml catalog number: 310107) (1 mg/ml)
3. Rat anti-mouse CD4 antibody (BD Biosciences/BD Pharmingen™, catalog number: 553727)
4. Rat anti-mouse CD8 antibody (BD Biosciences/BD Pharmingen™, catalog number: 553027)
5. BioMag suspension
6. Thymocytes

   Note: Whether add anti-CD4 antibody to do depletion or cell sorting is controversial because some researcher claimed that a minor subset of DN population, DN1, expresses low level of CD4.

Equipment

1. Imagnet magnetic separator (BD Biosciences/BD Pharmingen™, catalog number: 552311, Batch, catalog number: 0000038241)
   Note: Qiagen also offers magnetic separator suitable for different containers such as single-tube (catalog number: 36910), 12-tube (catalog number: 36912), 15 ml/50 ml tube (catalog number: 36935), and flask (catalog number: 36937) as well.

Procedure

A. Calculation
   1. For optimal results, use the most diluted cellular suspension possible. Typically, total cells are prepared at 2 x 10^7 total cells/ ml medium. Since any given cell source will have unique purification requirements, it is recommended to determine the optimal condition by individual user.
   2. QIAGEN suggests using 10-50 magnetic particles per cell (total cell population). However it has been proved that as few as 2-3 magnetic particles per total thymocytes works very efficiently. 1 mg/ml BioMag suspension contains 1 x 10^8 magnetic particles per milligram, which is equivalent to 1 x 10^8 magnetic particles per milliliter. Therefore, the volume of BioMag suspension required is 2.0-3.0 ml per 10^8 total thymocytes.

B. Prepare cell suspension
   Adjust the concentration at 2 x 10^7 total cells/ml sterile culture medium.

C. Primary antibody binds to cell surface antigen
   Add the appropriate amount of primary antibody to the cells and incubate for 30 min at 4 °C or on ice. In order to maximize the binding between the antigens and antibodies, invert the tube contains the cells and primary antibodies several times every 5 min. For optimal results, individual researcher needs to optimize the amount of primary antibody.

D. Wash BioMag particles
   1. During the procedure of binding, wash required amount of BioMag particles 2-3 times in appropriate sterile culture medium or buffer. First, suspend Biomag particles in original buffer which contains sodium azide, and use a magnetic separator (>20 megaoersted) to pull the magnetic particles to the side of the tube. Suck the original buffer carefully to removes the sodium azide preservative. Separation should be performed for 2-3 min using a magnetic separator.

2. Repeat the procedure with low protein (5% FCS) buffers are recommended to reduce nonspecific binding.

   Important: Do not centrifuge the BioMag suspension during wash steps. Centrifugation results in extensive aggregation and loss of binding activity.

E. Remove unbound primary antibody from the cells

Centrifuge at 200-300 *x g* for 5 min and suck the supernatant. Resuspend the cells in sterile medium and centrifuge at 200-300 *x g* for 5-10 min to wash the cells. Repeat wash step 1-2 times, using 1 ml sterile medium for each wash.

F. BioMag particles bind to primary antibody

Resuspend cells in appropriate volume of sterile medium and add the washed BioMag particles prepared in step 3. The final volume of sterile medium and BioMag particles should keep the cells at 2 x 10^7/ml. Incubate at 4 °C for 15 min. Swirl reaction vessel occasionally, or place eppendorf tubes on a rotating wheel during incubation.

Notes:

1. For optimal results, total volume should be ≥ 1ml (including BioMag suspension and cell volume). Where volumes <1 ml are used, additional medium or buffer should be added to a final volume of 1 ml.
2. Longer incubation is not recommended as magnetic particles may detach from the target cells as a result of cell surface changes over time.
3. Room temperature (15-25 °C) or 37 °C is optimal for some cell types and QIAGEN.
4. Recommends that the optimal cell sorting conditions be individually determined.

G. Separate target cells from other unwanted cells

Apply vessel to a magnetic separator for 10 min at 4 °C. Once separation is complete, carefully remove the supernatant without disturbing the pellet to another tube, the supernatant contains target cells after negative selection.

Notes:

1. A clear supernatant indicates that the separation is complete.
2. Separation must be performed with the vessel held vertically so that the pellet forms on the side of the flask or tube. This ensures unselected cells do not contaminate the magnetic pellet.
3. For optimal results, repeats step 7 once. The author strongly suggests repeating step 7 once with at least 15 min of separation because any contaminated magnetic particle will interfere with the following experiment especially cell sorting.

H. Count target cells

Centrifuge the supernatant at 200-300 *x g* for 5 min to pellet the cells, and resuspend the cells in fresh medium. Count the number of target cells for following experiment and calculate the depletion efficiency.

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References

1. Wang, H. C., Perry, S. S. and Sun, X. H. (2009). Id1 attenuates Notch signaling and impairs T-cell commitment by elevating Deltex1 expression. *Mol Cell Biol* 29(17): 4640-4652.

