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BioMag® and Cell Sorting

I. GENERAL PRODUCT DESCRIPTION

BioMag® products are a series of solid supports useful in magnetic separation. BioMag is an approximately 1.8 micron magnetic particle consisting of an iron oxide core with a silane coating. The particle surface is functionalized with amine or carboxyl groups for the covalent attachment of proteins, glycoproteins and other ligands with retention of biological activity. BioMag products are also supplied covalently attached to a number of second antibodies, such as goat anti-mouse, goat anti-rat and sheep anti-fluorescein as well as streptavidin, avidin and other binding proteins. All BioMag solid phases are superparamagnetic, i.e. they respond well to magnetic fields but do not themselves become magnetized. The inability to become permanently magnetized permits repeated magnetic extraction without magnetically induced aggregation. Efficient and rapid removal of BioMag particles from suspension is achieved through the application of an external magnetic field.

II. PRINCIPLE OF MAGNETIC CELL SORTING

BioMag is particularly suitable for cell isolations using either a *direct* or an *indirect* procedure. In the *direct* procedure, monoclonal or polyclonal antibodies to cell surface antigens are covalently attached to BioMag before incubation with the cell suspension. In the *indirect* procedure, the cells to be isolated are pretreated with the appropriate monoclonal or polyclonal antibodies and are subsequently magnetically immunoprecipitated with the appropriate magnetic second antibody. Variations of the *indirect* method include pretreating the cells to be isolated with either biotin-labelled antibodies or fluorescein-labelled antibodies and magnetically separating them with Magnetic Streptavidin or Magnetic Sheep anti-Fluorescein. The target cells magnetically labelled by either the *direct* or *indirect* method are easily isolated by applying a rare earth magnet directly against the side of the tube or tissue culture flask such that the cells are magnetically separated perpendicular to gravity. Most cell separations require a 5-10 minute magnetic separation.

III. MAGNETIC CELL SORTING GUIDELINES

One magnetic cell sorting protocol may not be applicable for all cell separations. The best way to start is by following the general guidelines offered here while referring to published protocols (See References, Data Sheet #528A). Each lab should then optimize antibody concentrations, incubations times, particle to cell ratios, etc. to achieve the desired results.

A. PREPARATION OF CELLS

Lymphocytes may be partially purified using a LeucoPREP™ Tube, ficoll gradient, nylon wool, or similar method. However, it may be possible to sort cells directly from whole blood and other sources. Since any particular cell source will have its own unique requirements for purification, procedures must be optimized. Ideally, cell concentrations should not exceed 5 million total cells per ml. The denser the mixture of cells, the more likely there will be nonspecific binding, clumping and trapping of cells. The investigator should work with the most dilute cellular suspension possible. Sorts or one million cells per ml are most common. If cells clump during washes with sterile media, it may be due to the release of DNA by necrotic cells. These clumps can be easily broken up with the use of 0.1% DNase in the cell medium.

B. BIOMAG PREPARATION

BioMag products are not claimed to be sterile and are supplied in buffers containing azide. Although not sterile, conjugated BioMag products are all prepared by procedures which essentially result in a low bioburden, i.e. glutaraldehyde conjugations. To remove azide and to prepare the magnetic particles for cell sorting, wash the magnetic particles 2-3 times in an appropriate sterile culture medium (or buffer) containing antibiotics such as penicillin, streptomycin or gentamycin. Washes MUST be performed using a BioMag magnetic separation unit. ***Do not centrifuge during wash steps.***

Media and buffers containing 5-10% protein are recommended to reduce possible non-specific binding. (Too much protein in the medium may inhibit the binding of particles to cells). Proteins such as human serum albumin, bovine serum albumin, fetal bovine serum, or milk solids may be used to lower non-specific binding.

C. POSITIVE AND NEGATIVE SELECTION USING BIOMAG

BioMag products may be used in both the positive and negative selection of cells. Depending upon antigen availability and the size of the target cell population, positive and negative cell sorting applications may require 20-80 magnetic particles per cell based on the total cell population. Multiple sorts may also be performed for both positive and negative selection. BioMag products contain approximately 5 x 10^8 magnetic particles per mg. Most BioMag products are supplied in 1mg BioMag/ml and 5 mg BioMag/ml concentrations. Since the particle to cell ratio is based upon the ***total*** cell population, the following sample calculation applies for both positive and negative selection.

1. Sample Calculation for Positive or Negative Selection

Consider a system in which there are 1 x 10^7 total cells and in which the target cell population is 30%. In this example we will use a ratio of 50 particles per total cell and the product, BioMag Goat anti-Mouse IgG which is supplied as a 1mg BioMag/ml preparation:

1 x 10^7 total cells x 50 particles per total cell = 5 x 10^8 magnetic particles required.

Since 1 mg/ml BioMag contain 5 x 10^8 particles per mg, which is equivalent to 5 x 10^8 magnetic particles per ml, the volume of washed BioMag required is:

$\frac{5 \times 10^8 \text{ magnetic particles required}}{5 \times 10^8 \text{ magnetic particles per ml}}$ = 1.0ml of BioMag Goat anti-Mouse IgG

D. INCUBATION GUIDELINES FOR POSITIVE AND NEGATIVE SELECTION

1. Incubations should be performed on ice or at 4°C to minimize patching, capping and phagocytosis. Also, cell viability may be best preserved by keeping the cells on ice. However, room temperature or even 37°C may be optimal for certain cell types. The ideal incubation temperature for sorting may vary with the application. Therefore, the investigator may wish to investigate other temperatures should low yield and/or cell viability become concerns.

2. Magnetic particles should be incubated with the cells for 15 to 30 minutes at 4°C. Long incubations are not recommended as magnetic particles may detach from the target cells as a result of cell surface changes over time. During incubation, gentle swirling of the reaction vessel at 10 minute intervals will keep the BioMag in suspension. (Continuous rotation is not recommended).

3. To reduce non-specific binding and prevent trapping, cell sorts should be performed in total volumes, which include the cell volume plus the BioMag volume, greater than or equal to 1ml. Additional media or buffer should be added to volumes less than 1ml to bring the volume to at least 1 ml.

E. SEPARATION GUIDELINES FOR POSITIVE AND NEGATIVE SELECTION

1. Magnetic separation must be performed perpendicular to gravity with the pellet formed on the side of the flask or tube. This technique is used to keep the unselected cells from contaminating the magnetic pellet due to gravity. The magnet used should have a strength greater than 20 megagauss Orstead. BioMag Separators (Cat.#84101S & 84102S) have been designed specifically for magnetically separating cells and are recommended.

2. Separation times of 5-10 minutes are generally sufficient for complete separations. A clear supernatant indicates that the separation is complete.

3. Once separation is complete, the supernatant should be removed without disturbing the magnetic pellet. For this reason, vacuum aspiration is not recommended.

F. GENERAL PROCEDURE FOR THE INDIRECT METHOD FOR ISOLATING LYMPHOCYTES

This procedure is for negative or positive cell selection which is best carried out in sterile tissue culture tubes or flasks. The following is an example of a two step protocol which uses BioMag Goat anti-Mouse IgG (Cat.#84340). The target population is assumed to be 20% of the total. All steps are done at 4°C. *The example uses a particle to total cell ratio of 50:1 for BioMag Goat anti-Mouse IgG which is supplied at a concentration of 1mg/ml.*

1. Approximately 10 million total cells are placed in a tube in 10 ml of RPMI with 5% fetal bovine serum and antibiotics.

2. Wash 1ml of BioMag Goat anti-Mouse IgG three times in 1ml of sterile medium containing antibiotics. Use a magnet to pull the magnetic particles to the side of the tube and shake vigorously to resuspend the magnetic particles during washing. Resuspend in 1ml of sterile medium.

3. Depending upon the source of the antibody and the manufacturer's recommendations, 5-20 micrograms of monoclonal antibody per one million target cells is typically needed. Add the appropriate amount of monoclonal antibody to the washed 1ml of BioMag Goat anti-Mouse IgG from step 2 and mix. Incubate at 4°C for 20 minutes.

4. Magnetically separate the BioMag Goat anti-Mouse IgG/antibody complex and wash three times with 1ml of sterile medium. Resuspend in 1 ml of sterile medium.

5. Add the 1ml of washed BioMag Goat anti-Mouse IgG/antibody complex to the 10 ml of cells. (The particle-to-total-cell-ratio is 50:1). Gently swirl the cell/particle mixture to resuspend the cells. The cells are incubated for 20 minutes at 4°C. Swirl the cell/particle suspension every 10 minutes to promote attachment. Magnetically separate for 10 minutes and save the supernatant for a negative selection or save the magnetic pellet for a positive selection.

6. Centrifuge and resuspend the cells in fresh medium for negatively selected cells. For positively selected cells, refer to "Removal of Cells from BioMag After Positive Selection," Section H.

G. GENERAL PROCEDURE FOR THE DIRECT METHOD OF SEPARATING LYMPHOCYTES

This procedure is for negative or positive cell selection which is best carried out in sterile tissue culture tubes or flasks. The following is an example of a one step protocol which uses BioMag magnetic particles covalently attached to CD8 monoclonal antibody (Cat.#84708). The target population is assumed to be 25% of the total. All steps are done at 4°C. *The example uses a particle to total cell ratio of 100:1 for BioMag anti-CD8 which is supplied at a concentration of 1mg/ml.*

1. Deliver one ml of approximately 1 x 10^6 cells in RPMI with 5% fetal bovine serum and 1% penicillin-streptomycin into an appropriate tube.

2. Wash 0.2ml of BioMag anti-CD8 magnetic particles three times in 0.2ml of sterile medium containing antibiotics. Use a magnet to pull the magnetic particles to the side of the tube and shake vigorously to resuspend the magnetic particles during washing.

3. Add 0.2 ml of washed BioMag anti-CD8 magnetic particles to the cells and swirl to mix the cells with the BioMag. (The particle-to-cell-ratio in this example is 100 particles per cell based on the total cell population).

4. Incubate the cells with the BioMag anti-CD8 for 20-30 minutes. Swirl the cell/particle suspension once every ten minutes to promote attachment. Magnetically separate the supernatant for 10 minutes twice and save the supernatant for a negative selection or save the magnetic pellet for a positive selection.

5. Centrifuge and resuspend the negatively selected cells in fresh medium for negative selection. For positive selection, refer to "Removal of Cells From BioMag After Positive Selection," Section H.

H. REMOVAL OF CELLS FROM BIOMAG AFTER POSITIVE SELECTION

1. Methods for detaching magnetic particles from cells after separation include culturing cells for up to 48 hours during which magnetic particles fall away from the cells due to cell surface turnover (See References Data Sheet #528A, Pricop et al) or using a protease, such as chymopapain, to break the antigen-antibody bond. Each of these procedures has limitations. Not all particles may detach from cells during culturing and the use of a protease may damage cells. Depending upon the application, it may not be necessary to remove the cells from the BioMag particles. BioMag particles are only 1µm in size and can be successfully used in flow cytometry equipment as they will not jam the equipment and are distinguishable from cells. Alternatively, negative selection should be considered.

*For BioMag Cell Sorting References, please call customer service and ask for Technical Data Sheet #528A or visit our web site at **www.polysciences.com** and access the technical library to view data sheet #528A