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BioMag® and Cell Sorting

DESCRIPTION

BioMag® products are a series of solid supports useful in magnetic separation. BioMag® is an approximately 1.5µm magnetic particle consisting of an iron oxide core with 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 with covalently bound with a number of secondary 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.

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-labeled antibodies or fluorescein-labeled antibodies and magnetically separating them with BioMag® Streptavidin or BioMag® Sheep anti-Fluorescein. The target cells magnetically labeled 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.

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 Technical Data Sheet (TDS) 528A, *BioMag® and Cell Sorting References*). Each lab should then optimize antibody concentrations, incubation 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 the 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 five 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. 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 sodium azide. Although not sterile, conjugated BioMag® products are all prepared by procedures that essentially result in a low bioburden, i.e. glutaraldehyde conjugations. To remove sodium 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 magnetic separation unit. *Do not centrifuge during wash steps.*

Media and buffers containing 5-10% protein are recommended to reduce possible nonspecific 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 nonspecific 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 1 x 10^8 magnetic particles per mg. Most BioMag® products are supplied in 1mg BioMag®/ml and 5mg 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.

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® 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 1mg/ml BioMag®* contains 1 x 10^8 particles per mg, which is equivalent to 1 x 10^8 magnetic particles per ml, the volume of washed BioMag® required is:

> 1 x 10^8 magnetic particles required = 5.0ml of BioMag® anti-Mouse

IgG 1 x 10^8 magnetic particles per ml.
** The concentration of BioMag® products varies and should be confirmed by checking the Certificate of Analysis before calculations are performed.*

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 cells for 15-30 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® is suspension. (Continuous rotation is not recommended.)
3. To reduce nonspecific 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 1ml.

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 Oerstead.
2. Separation times of 5-10 minutes are generally sufficient for complete separations. A clear supernatant indicates 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. 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 that uses BioMag® 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® anti-Mouse IgG, which is supplied at a concentration of 1mg/ml.

1. Approximately 10 million total cells are placed in a tube of 10ml of RPMI with 5% fetal bovine serum and antibiotics.
2. Wash 1mL of BioMag® anti-Mouse IgG 3 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 or vortex to resuspend the magnetic particles during washing. Resuspend in 1ml of sterile medium.
3. Depending on the source of the antibody and the manufacturer's recommendations, 5-20µg of monoclonal antibody per one million target cells is typically needed. Add the appropriate amount of monoclonal antibody to the washed 1mL of BioMag® anti-Mouse IgG from Step 2 and mix. Incubate at 4˚C for 20 minutes.
4. Magnetically separate the BioMag® anti-Mouse IgG / antibody complex and wash 3 times with 1ml of sterile medium. Resuspend in 1ml of sterile medium.
5. Add the 1ml of washed BioMag® anti-Mouse IgG / antibody complex to the 10ml of cells. (The particle to total cell ratio is 50:1.) Gently swirl the cell / particle mixture to resuspend the cells. Incubate the cells 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 *Section H: Removal of Cells from BioMag® After Positive Selection.*

G. 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 that uses BioMag® magnetic particles covalently coated with CD8 monoclonal antibody (Cat. #85008). 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 1ml 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 3 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 0.2mL of washed BioMag® anti-CD8 magnetic particles to the cells and swirl to mix the cells with the BioMag®. (The particle to total cell ratio in this example is 100 particles per cell based on the total cell population.)
4. Incubate the cells with BioMag® anti-CD8 for 20-30 minutes. Swirl the cell / particle suspension every 10 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 cells in fresh medium for negatively selected cells. For positively selected cells, refer to *Section H: Removal of Cells from BioMag® After Positive Selection.*

H. Removal of Cells from BioMag® After Positive Selection

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 TDS 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 BioMag® particles. BioMag® particles are only 1-2µm in size and can be successfully used in flow cytometry equipment, as they will not clog the equipment and are distinguishable from cells. Alternatively, negative selection should be considered.

** For BioMag® Cell Sorting References, please see TDS 528A.

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