



BioMag[®] Sheep Anti-Fluorescein IgG

BioMag[®] suspensions are a series of cell-sorting products containing superparamagnetic particles. BioMag particles are irregularly shaped, with an average diameter of 1.6 μm , and consist of an iron oxide core with a silane coating. The surface of the particle is coated with amine or carboxyl groups, facilitating the covalent attachment of proteins, glycoproteins, secondary antibodies, and other ligands, with retention of biological activity. The particles' irregular shape provides a large surface area, ensuring a high binding efficiency.

BioMag particles are superparamagnetic — they respond to magnetic fields but do not retain magnetic properties upon removal of the magnetic field. This inability to become magnetized permits magnetic extraction without magnetically induced aggregation. Rapid and efficient removal of BioMag particles from suspension is achieved by the application of an external magnetic field.

Depending on antigen availability and the total cell population, cell sorting may require between 10 and 50 magnetic particles per cell. Multiple cell sorts can also be performed. BioMag suspensions contain approximately 1×10^8 magnetic particles per milligram, and are supplied in concentrations of 1 or 5 mg/ml. The particle-to-cell ratio is based on the total cell population, as demonstrated in the following sample calculation.

Sample variables

- A system with 1×10^7 total cells in 10 ml (i.e., 10^6 cells/ml)
- 10–50 magnetic particles per cell (total cell population)
- BioMag suspension with 1 mg BioMag particles/ml

Therefore, 1×10^7 cells \times 10–50 particles per cell = $1\text{--}5 \times 10^8$ magnetic particles are required per 10 ml cells.

1 mg/ml BioMag suspension contains 1×10^8 magnetic particles per milligram, which is equivalent to 1×10^8 magnetic particles per milliliter. Therefore, the volume of washed BioMag suspension required is 1.0–5.0 ml per 10 ml cells.

Applications

BioMag Sheep Anti-Fluorescein IgG is a standard BioMag particle coated with polyclonal sheep anti-fluorescein IgG antibodies and is highly suited for use in cell sorting methods where a fluorescein IgG antibody is used as a primary antibody. BioMag Sheep Anti-Fluorescein IgG can be used to separate the cells of interest from a heterogeneous cell population using negative depletion. BioMag Sheep Anti-Fluorescein IgG can also be used as a secondary antibody in enzyme immunoassays and radioassays that utilize a fluorescein IgG primary monoclonal antibody.

Specifications

Form	Magnetic particle suspension (1 mg/ml) in phosphate-buffered saline containing EDTA and 0.08% sodium azide.
BioMag concentration	1 mg/ml
Binding capacity	1 ml (1 mg) BioMag Sheep Anti-Fluorescein IgG binds >10 µg fluoresceinated albumin.
Storage and stability	BioMag Sheep Anti-Fluorescein IgG can be stored at 2–8°C until the expiration date. Do not freeze. Do not dry. Centrifugation should only be used if it is the last step of a procedure, i.e., if resuspension of the BioMag particles is not required. Freezing, drying, and centrifugation result in extensive aggregation of the BioMag particles and a loss of binding activity.
Safety information	When working with chemicals always wear a suitable lab coat, disposable gloves, and protective goggles. For more information please consult the appropriate material safety data sheet, available online at www.qiagen.com/ts/msds.asp

Preparation of cells

Lymphocytes can be partially purified using a LeucoPREP® Tube, Ficoll® gradient, nylon wool, or other similar method, although it may be possible to sort cells directly from whole blood or other sources. Since any given cell source will have unique purification requirements, we recommend determining the optimal conditions. For optimal results we recommend using the most dilute cellular suspension possible. Cell concentrations should not exceed 5×10^6 total cells per ml, since higher cell density increases the risk of nonspecific binding.

General protocol

This protocol is a general guide to cell separation using the BioMag System, and is applicable in most cases. We recommend optimizing the conditions for your cells. A linear scaleup or -down of volumes and cell numbers is possible.

1. **Wash required amount of BioMag particles 2–3 times in appropriate sterile culture medium or buffer. Resuspend in 1 ml sterile medium. Use a magnetic separator (>20 megaoersted) to pull the magnetic particles to the side of the tube.**

Washing of BioMag particles removes the sodium azide preservative. Low protein (5–10%) media and buffers are recommended to reduce nonspecific binding.

Washes must be performed using a magnetic separation unit. Appropriate magnets are available from QIAGEN; see ordering information.

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

2. **Prepare 10^7 total cells in 10 ml sterile culture medium (10^6 cells/ml). Store as appropriate for sample type until step 6.**

3. **Add the appropriate amount of monoclonal antibody to the washed BioMag suspension from step 1 and mix by inversion.**

5–20 μ g monoclonal antibody per 10^6 target cells is usually sufficient depending on the monoclonal antibody used.

4. **Incubate at 4°C for 20 minutes to allow binding of the monoclonal antibody to the BioMag particles.**

5. **Separate the BioMag particles/antibody complex using a magnetic separator. Wash 3 times using 1 ml sterile medium for each wash. Resuspend in 1 ml sterile medium.**

6. **Add 1 ml washed BioMag particles/monoclonal antibody mix to 10 ml cells prepared in step 2. Gently swirl the vessel to ensure complete resuspension.**

Note: We recommend sorting cells in total volumes ≥ 1 ml (including BioMag suspension and cell volume). Where volumes < 1 ml are used, additional media or buffer should be added to a final volume of 1 ml.

7. **Incubate at 4°C for 15–30 minutes. Swirl reaction vessel gently at 10 minute intervals during incubation. Do not rotate continuously.**

Longer incubation and continuous rotation are not recommended as magnetic particles may detach from the target cells as a result of cell surface changes over time.

Room temperature (15–25°C) or 37°C are optimal for some cell types and QIAGEN recommends that the optimal cell sorting conditions be individually determined.

8. **Apply vessel to a magnetic separator for 5–10 minutes.**

Note: A clear supernatant indicates that the separation is complete. 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.

9. **Once separation is complete, remove the supernatant without disturbing the pellet.**

10. **Centrifuge the supernatant at 200–300 x g for 5–10 minutes to pellet the cells, and resuspend the cells in fresh medium.**

Ordering Information

Product	Contents	Cat. No.
BioMag Sheep Anti-Fluorescein IgG (50 ml)	BioMag sheep anti-fluorescein IgG secondary antibody suspension (1 mg/ml)	310704
BioMag Sheep Anti-Fluorescein IgG (500 ml)	BioMag sheep anti-fluorescein IgG secondary antibody suspension (1 mg/ml)	310707
Accessories		
Single Tube Magnet	Magnet for separating magnetic particles in a 1.5 ml or 2 ml tube	36910
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
15 ml/50 ml Tube Magnet	Magnet for separating magnetic particles in 5 x 15 ml and 3 x 50 ml tubes	36935
Flask Magnet	Magnet for separating magnetic particles in a cell culture flask	36937

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