BioMag® Goat Anti-Rat 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 x 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 x 10^7 total cells
- 10–50 magnetic particles per cell (total cell population)
- BioMag suspension with 1 mg BioMag particles/ml

Therefore, 1 x 10^7 cells x 10–50 particles per cell = 1–5 x 10^8 magnetic particles are required.

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 1.0–5.0 ml per 10^7 cells.

Applications

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

Specifications

Form	Magnetic particle suspension (1 mg/ml) in phosphate-buffered saline (PBS) containing EDTA and 0.08% sodium azide.
BioMag concentration	1 mg/ml
Binding capacity	1 ml (1 mg) BioMag Goat Anti-Rat IgG binds >0.10 mg rat IgG. The antibody will react with heavy chains on rat IgG and light chains on all rat immunoglobulins.
Storage and stability	BioMag Goat Anti-Rat 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 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.

General protocol

The protocol for the indirect method of cell separation 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. Guidelines for the direct method of cell separation are included. We recommend using the indirect method, since less primary antibody is required.

Protocol for indirect method of cell separation

1. **Wash required amount of BioMag particles 2–3 times in appropriate sterile culture medium or buffer. Resuspend in 500 µl 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% FCS or 0.5% BSA) media and buffers are recommended to reduce nonspecific binding. If coagulation of the cells is a problem, include an anticoagulant, such as 2 mM EDTA in the sterile culture medium or buffer. Separation should be performed for 2–3 min using a magnetic separator. Appropriate magnets are available from QIAGEN; see Ordering Information.
   Important: Do not centrifuge the BioMag suspension during wash steps. Centrifugation results in extensive aggregation and loss of binding activity.

2. **Prepare 10^7 total cells in 100–500 µl sterile culture medium.**

3. **Add the appropriate amount of primary antibody to the cells and incubate for 20–30 min at 4°C.**
   For optimal results, we recommend optimizing the amount of primary antibody. As little as 0.1–1.0 µg primary antibody per 10^7 target cells may be sufficient for effective selection of target cells using this protocol.

4. **Remove unbound primary antibody from the cells by centrifugation at 200–300 x *g* for 5–10 min. Resuspend the cells in 1 ml 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.**

5. **Resuspend cells in 1 ml sterile medium and add the washed BioMag particles prepared in step 1.**
   Note: We recommend sorting cells in total volumes ≥ 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.

6. **Incubate at 4°C for 15–30 min. Swirl reaction vessel occasionally, or place eppendorf tubes on a rotating wheel during incubation.**
   Longer incubation is 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.

7. **Apply vessel to a magnetic separator for 5–10 min.**
   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.

8. **Once separation is complete, carefully remove the supernatant without disturbing the pellet.**
   For optimal results, we recommend repeating step 7.

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

Guidelines for the direct method of cell separation

Direct binding of primary antibodies to the BioMag particles may be useful for some applications. Washed BioMag particles (see step 1 of the indirect method for cell separation) should be incubated with the appropriate amount of primary antibody at 4°C for 20 min in 500 µl of sterile medium or buffer. Considerably larger amounts of primary antibody are required compared with the indirect method of cell separation. After separation (using a suitable magnetic separator), the BioMag particles/antibody complex should be washed 3 times using 1 ml sterile medium for each wash. Afterwards, the protocol for the indirect method of cell separation should be followed from step 5.

Ordering Information

Product	Contents	Cat. No.
BioMag Goat Anti-Rat IgG (50 ml)	BioMag goat anti-rat IgG secondary antibody supension (1 mg/ml)	310104
BioMag Goat Anti-Rat IgG (500 ml)	BioMag goat anti-rat IgG secondary antibody supension (1 mg/ml)	310107
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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