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invitrogen bead separations

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Dynabeads® Rat anti-Mouse IgG1

For research use only

1. PRODUCT DESCRIPTION

1.1 Intended Use

Dynabeads Rat anti-Mouse IgG1 in combination with primary mouse IgG1 antibodies are ideal for depletion or positive isolation of cells from different species (e.g. human, rat) depending on the specificity of the primary antibody. Cells can be directly isolated from any sample such as whole blood, bone marrow, MNC suspensions or tissue digests.

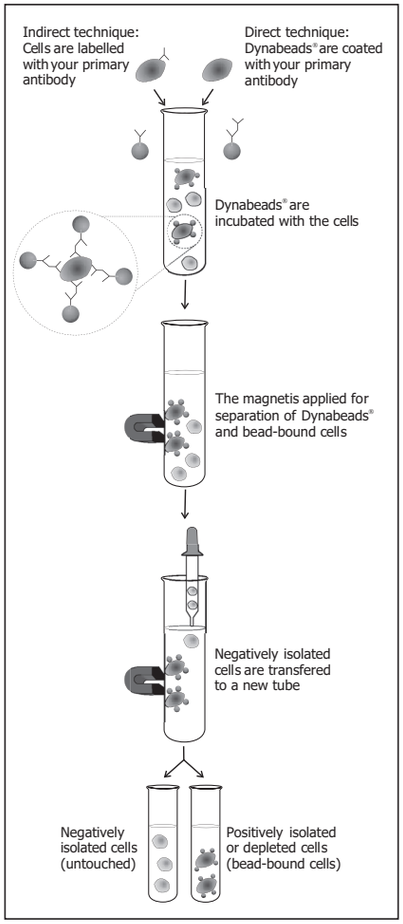

Fig. 1: Cell isolation using indirect or direct technique.

1.2 Principle of Isolation

The primary mouse IgG1 antibody is either added to the cell sample (indirect technique) or pre-coated onto the beads (direct technique) prior to cell isolation.

Dynabeads are then mixed with the cell sample in a tube. The Dynabeads will bind to the target cells during a short incubation, and then the bead-bound cells are separated by a magnet.

- **Positive isolation** – discard the supernatant and use the bead-bound cells for downstream applications (e.g. molecular analysis or cell culture).
- **Depletion** – discard the bead-bound cells and use the remaining, untouched cells for any application.

1.3 Description of Materials

Dynabeads Rat anti-Mouse IgG1 are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with monoclonal rat anti-mouse IgG1 antibodies. The antibody coated onto Dynabeads recognises mouse gamma1 heavy chain of immunoglobulin. No cross-reactivity to human IgG or IgM.

Materials Supplied

- **5 ml Dynabeads Rat anti-Mouse IgG1**

  4 x 10^8 beads/ml in phosphate buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide (NaN_3).
- **This product will process up to 2 x 10^9 cells**

Additional Materials Required

- Magnet: (Dynal MPC™) - MPC-S for 20 µl to 2 ml samples, MPC-L for 1-8 ml samples, MPC-15 for 1-15 ml samples and MPC-50 for 15-50 ml samples.
- Mixer allowing both tilting and rotation.
- Buffer 1: PBS w/0.1% BSA and 2 mM EDTA, pH 7.4.
- Mouse IgG1 antibodies.

Important Notes:

BSA can be replaced by human serum albumin (HSA) or FCS.

EDTA can be replaced by sodium citrate.

PBS containing Ca^{2+} or Mg^{2+} is not recommended.

2. PROTOCOLS

2.1 Dynabeads Washing Procedure

Dynabeads should be washed before use.

1. Resuspend the Dynabeads in the vial.
2. Transfer the desired volume of Dynabeads to a tube.
3. Add the same volume of Buffer 1, or at least 1 ml, and mix.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed Dynabeads in the same volume of Buffer 1 as the initial volume of Dynabeads.

2.2 Sample Preparation

Cells can be directly isolated from any sample such as whole blood, bone marrow, MNC or tissue digests.

Please visit www.invitrogen.com/samplepreparation for a list of recommended sample preparation procedures.

2.3 Critical Steps for Cell Isolation

- Use a mixer that provides tilting and rotation of the tubes to ensure Dynabeads do not settle at the bottom of the tube.
- When incubating Dynabeads and cells, the incubation temperature must be 2 - 8°C to reduce phagocytic activity and other metabolic processes.
- Never use less than 25 µl (1 x 10^7) Dynabeads per ml cell sample and at least 4 Dynabeads per target cell.

Table 1: Volume of Dynabeads added per ml of cell sample. The volumes can be scaled up as required.

	Positive isolation	**Depletion**
Sample volume (1 x 10^7 cells/ml*)	1 ml Max 2.5 x 10^6 target cells	1 ml Max 2.5 x 10^6 target cells
Volume of Dynabeads	25 µl	50 µl

* If the concentration of cells is increased or the target cell concentration exceeds 2.5 x 10^6, the Dynabeads volume must be increased accordingly. Cell concentration can be up to 1 x 10^8 cells per ml.

2.4 Cell Isolation - Indirect Technique

2.4.1 Labelling Cells with Mouse IgG1 Antibodies

- Use approximately 1 µg antibody per 10^6 target cells.
- Recommended cell concentration: 1 x 10^7 cells/ml.

1. Add mouse IgG1 antibodies to the cell suspension. Mix well and incubate for 20 min at 2-8°C.
2. Wash the cells by adding 2 ml Buffer 1 per 1 x 10^7 cells and centrifuge at 300 x g for 8 min. Discard the supernatant.
3. Resuspend the cells in Buffer 1 at 1 x 10^7 cells/ml.

2.4.2. Isolation or Depletion of Cells

1. Add Dynabeads to the prepared sample according to table 1.
2. Incubate for 20 min (positive isolation) or 30 min (depletion) at 2 - 8°C with gentle tilting and rotation.
3. Double the volume with Buffer 1 to limit trapping of unbound cells (optional).
4. Place the tube in a magnet for 2 min.
5. Depletion: Transfer the supernatant containing the unbound cells to a fresh tube for further experiments.
6. Positive isolation: Discard the supernatant and gently wash the bead-bound cells 4 times, using the following procedure:
   i) Add 1 ml Buffer 1 per 1 x 10^7 Dynabeads.
   ii) Place the tube in the magnet for 1 min and discard the supernatant.
7. Resuspend the cells in buffer/medium for downstream application.

2.5 Cell Isolation - Direct Technique

2.5.1 Pre-coating Dynabeads

- Use 0.5 – 1.5 µg mouse IgG1 antibodies per 25 µl (1 x 10^7) Dynabeads.

1. Transfer washed Dynabeads to a tube.
2. Add antibodies.
3. Incubate for ≥ 30 min with gentle tilting and rotation.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Wash the beads twice using 2 ml of Buffer 1.
6. Remove the tube from the magnet and resuspend the washed Dynabeads in the same volume of Buffer 1 as the initial volume of Dynabeads.

2.5.2 Isolation or Depletion of Target Cells

1. Add Dynabeads to the prepared sample according to table 1.
2. Incubate for 20 min (positive isolation) or 30 min (depletion) at 2 - 8°C with gentle tilting and rotation.
3. Double the volume with Buffer 1 to limit trapping of unbound cells (optional).
4. Place the tube in a magnet for 2 min.
5. Depletion: Transfer the supernatant containing the unbound cells to a fresh tube for further experiments.
6. Positive isolation: Discard the supernatant and gently wash the bead-bound cells 4 times, using the following procedure:
   i) Add 1 ml Buffer 1 per 1 x 10^7 Dynabeads.
   ii) Place the tube in the magnet for 1 min and discard the supernatant.
7. Resuspend the cells in buffer/medium for downstream application.

3. TECHNICAL ADVICE

3.1 Indirect versus Direct Technique

Use the indirect technique when:

- A cocktail of mouse IgG1 antibodies is used.
- Very high depletion efficiency is needed.
- The affinities of mouse IgG1 antibodies are low.
- The cells express low number of target antigens.
- The direct technique gives unsatisfactory purity.

The direct technique may be used if:

- The affinity of the primary antibody is high.
- The cells express a high number of target antigens.
- A stock preparation of primary coated Dynabeads is desired.

3.2 Antibody Selection

The choice of primary antibody is the most important factor for successful cell isolation. Note that some antibodies may show reduced antigen-binding efficiency when coated onto beads (direct technique), even though the antibody shows good results in other immunological assays.

3.3 Labelling Cells with Mouse IgG1 Antibodies

- Titrate the primary antibody to optimise the amount used.
- To avoid non-specific binding of cells (e.g. monocytes, B cells), add aggregated IgG to block Fc receptors prior to adding the primary antibodies.
- Excess antibody must be removed by washing before cell isolation.

3.4 Isolation and Depletion of Target Cells

- Remove density gradient media (e.g. Ficoll): Wash cells prior to adding mouse IgG1 antibodies or Dynabeads.
- Remove soluble factors in serum: Serum may contain soluble factors (e.g. antibodies or cell surface antigens), which can interfere with the cell isolation protocol. Washing the cells once may reduce this interference.

4. GENERAL INFORMATION

Invitrogen Dynal AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

Storage/Stability

This product is stable until the expiry date stated on the label when stored unopened at 2-8°C.

Store opened vials at 2-8°C and avoid bacterial contamination.

Keep Dynabeads in liquid suspension during storage and all handling steps, as drying will result in reduced performance. Resuspend well before use.

Technical Support

Please contact Invitrogen Dynal for further technical information (see contact details).

Warning And Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated.

Follow appropriate laboratory guidelines.

This product contains 0.02% sodium azide as a preservative, which is cytotoxic. **Avoid pipetting by mouth!** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide build up.

Certificate of Analysis (CoA) is available upon request.

Material Safety Data Sheet (MSDS) is available at http://www.invitrogen.com.

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