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invitrogen bead separations

Cat. no. 110.47

Rev. no. 002

Dynabeads® Biotin Binder

For research use only

1. PRODUCT DESCRIPTION

1.1 Intended Use

Dynabeads Biotin Binder in combination with biotinylated antibodies are ideal for depletion or positive isolation of cells from any species depending on the specificity of the antibody. Other biotinylated molecules (e.g. peptides/proteins, lectins or nucleic acids) may also be used depending on the target. Cells can be directly isolated from any sample, such as whole blood, bone marrow, mononuclear suspensions or tissue digests.

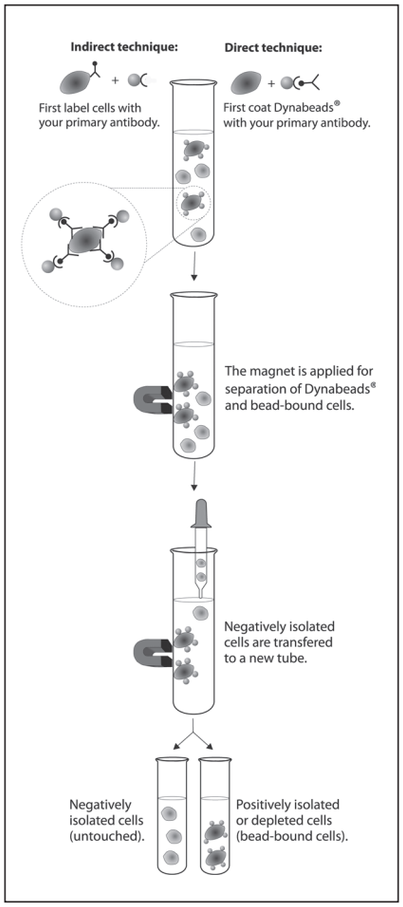

Fig. 1: Cell isolation using indirect or direct technique. The primary antibody is biotinylated prior to use.

Note:

If you want to perform positive cell isolation with biotinylated antibodies followed by bead detachment, please use CELLection™ Biotin Binder (Cat. no. 161.02).

If you want to use biotinylated ligands for molecular applications such as protein purifications, DNA/RNA binding protein isolations, preparing single-stranded templates etc., please see www.invitrogen.com for other streptavidin coated beads.

1.2 Principle of Isolation

Either add the biotinylated antibody to the cell sample (indirect technique) or pre-coat onto the beads (direct technique) prior to cell isolation. Mix the Dynabeads with the cell sample in a tube. The Dynabeads will bind to the target cells during a short incubation, and then separate the bead-bound cells by a magnet (see fig.1).

- Positive Isolation - discard the supernatant and use the bead-bound cells for downstream applications.
- Depletion - discard the bead-bound cells and use the remaining, untouched cells for any application.

1.3 Description of Materials

Dynabeads Biotin Binder are uniform, superparamagnetic polystyrene beads (2.8 µm diameter) coated with recombinant streptavidin. The streptavidin coated onto Dynabeads will bind most biotinylated ligands. Unwanted binding of cells to streptavidin via lectin-like receptors or other adhesive receptors is avoided since the recombinant streptavidin contains neither sugar nor the RYD-sequence.

Materials Supplied

5 ml Dynabeads Biotin Binder

4 x 10^8 beads/ml in phosphate buffered saline (PBS), pH 7.4, containing 0.1% BSA and 0.02% sodium azide (NaN_3).

This product will process up to 2 x 10^9 cells

Additional Materials Required

- Biotinylated ligand (e.g. antibodies, peptides/proteins, lectins or nucleic acids).
- Magnet (Dynal MPC™):
  See www.invitrogen.com/magnets-selection for magnet recommendations.
- Mixer allowing both tilting and rotation.
- Buffer 1: PBS w/0.1% BSA and 2 mM EDTA, pH 7.4.

Important Notes:

Serum or serum albumin fractions may contain free biotin and should not be used. Dynal recommends Bovine Serum Albumin fraction V from Sigma (cat. no. A4503 or equivalent), which does not contain free biotin.

Do not use culture media as it may contain free biotin.

BSA can be replaced by other biotin-free blocking proteins or 1 mg/ml pluronic (F68).

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 2 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 (step 2).

2.2 Sample Preparation

2.2.1 Whole Blood and Buffy Coat

Whole blood and buffy coat may be washed before use to remove interfering factors.

1. Dilute the whole blood or buffy coat in Buffer 1 (1+2).
2. Centrifuge at 600 x g for 10 min at RT (18-25°C).
3. Discard the plasma fraction/upper layer.
4. Resuspend to the original volume in Buffer 1 (2-8°C).

2.2.2 MNC Preparation from Whole Blood, Buffy Coat or Bone Marrow

1. Resuspend at 1 x 10^7 cells per ml in Buffer 1 (2-8°C).

Please contact technical centre for a list of recommended human and mouse 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 Biotinylated Antibodies or Ligands

Label cells with biotinylated antibody as recommended by the manufacturer. If necessary, optimize incubation time, antibody titer and cell concentration for best signal to noise ratio.

Use approximately 1 µg biotinylated antibody per 10^6 target cells (if no other recommendation is stated) and ≥ 1 x 10^7 cells/ml.

1. Add primary antibody to the cell suspension and mix.
2. Incubate for 10 min at 2-8°C.
3. 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.
4. Resuspend the cells in Buffer 1 at 1 x 10^7 cells per ml.
5. Proceed to Isolation or Depletion of Cells (2.4.2).

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. Optional: double the volume with Buffer 1 to limit trapping of unbound cells.
4. Place the tube in a magnet for 3 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 2 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 biotinylated antibody per 25 µl (1 x 10^7) Dynabeads. It is recommended to titrate the amount of antibody.

1. Transfer washed Dynabeads to a tube.
2. Add biotinylated antibodies.
3. Incubate for > 30 min with gentle tilting and rotation.
4. Place the tube in a magnet for 2 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 the pre-coated Dynabeads to the cells 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. Optional: double the volume with Buffer 1 to limit trapping of unbound cells.
4. Place the tube in a magnet for 3 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 2 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 biotinylated antibodies is used.
- Very high depletion efficiency is needed.
- The affinities of biotinylated monoclonal 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 biotinylated antibody is high.
- The cells express a high number of target antigens.
- A stock preparation of pre-coated Dynabeads is desired.

3.2 Antibody Selection

The choice of biotinylated 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.

Some antibodies may be delivered in buffers containing high levels of free biotin. To be able to pre-coat the Dynabeads (direct technique), the free biotin needs to be removed (e.g. dialysis or spin column with MW 10, 000 cut-off).

3.3 Labelling Cells with Biotinylated Antibodies

- To avoid non-specific binding of cells (e.g. monocytes, B cells), add aggregated IgG to block Fc receptors prior to adding the biotinylated antibody.
- 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 biotinylated 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 'www.invitrogen.com'.

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Fax (760) 602-6500.
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SPEC-05724