invitrogen
by life technologies

Dynabeads® Biotin Binder Kit

Catalog no. 11047 **Store at 2˚C to 8˚C**

Rev. Date: June 2012 (Rev. 003)

Product Contents

Cat. no	Volume
11047	5 mL

Maximum product capacity

MNC*: ~2 × 10^9 cells

Whole blood/buffy coat: ~200 mL

* Note: If using the product for negative isolation of multiple cell types simultaneously, the bead volume used is higher, thus giving a lower product capacity (see Table 1 and 2).

Dynabeads® Biotin Binder contains 4 × 10^8 beads/mL in phosphate buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative.
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

Dynabeads® Biotin Binder in combination with primary biotinylated antibodies are ideal for depletion or positive isolation of cells from different species (e.g. mouse, human), 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. Other biotinylated molecules (e.g. peptides/proteins, lectins or nucleic acids) may also be used depending on the target.

The primary biotinylated antibodies are either added to the cell sample (indirect technique) or pre-coated onto the beads (direct technique) prior to cell isolation (fig. 1). Dynabeads® are then mixed with the cell sample in a tube. The Dynabeads® 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. isolation of proteins or nucleic acids [NA] or cell culture).
Note: For positive isolation of cells for downstream cellular applications, or for use in flow cytometry, bead-free cells are required. For these applications, use the equivalent CELLection™ Biotin Binder Kit (enzymatic cleavage of a DNA-linker that releases the cells from the Dynabeads®).

For isolation of phagocytic cells use Dynabeads® FlowComp™ Flexi (can be used with lower temperature that is necessary to reduce phagocytic activity).

Depletion/negative isolation – Discard the bead-bound cells and use the remaining bead-free and untouched cells for any application. If different biotinylated antibodies are used to deplete several cell types simultaneously (negative isolation) to obtain untouched cells, use the indirect technique.

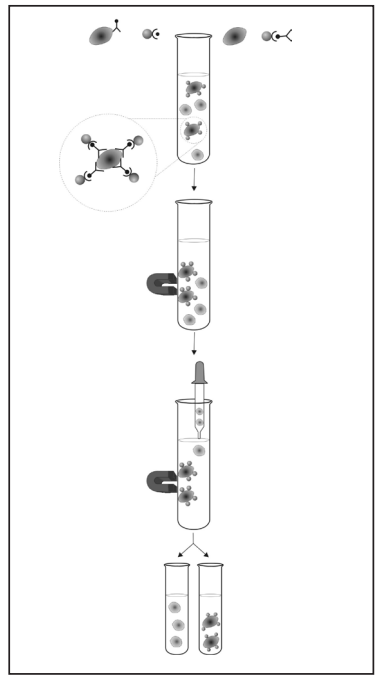

Figure 1: Cell isolation using indirect or direct technique

Required Materials

- Magnet (DynaMag™)
  See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Buffer: Ca^{2+} and Mg^{2+} free PBS supplemented with 0.1% bovine serum albumin (BSA) and 2 mM EDTA, pH 7.4.
  Note: BSA can be replaced by human serum albumin (HSA) or fetal calf serum (FCS). EDTA can be replaced by sodium citrate
- Biotinylated antibodies.

General Guidelines

- Visit www.lifetechnologies.com/samplepreparation for recommended sample preparation procedures.
- Use a mixer that provides tilting and rotation of the tubes to ensure that Dynabeads® do not settle at the bottom of the tube.
- 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, even though the antibody shows good results in other immunological assays.
- 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.
- Wash cells prior to adding biotinylated antibodies or Dynabeads® to remove density gradient media (e.g. Ficoll) or soluble factors in serum (e.g. antibodies or cell surface antigens), which can interfere with the cell isolation protocol.
- This product should not be used with the MPC™-1 magnet (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Never use less than recommended volume of Dynabeads®.
- Carefully follow the recommended volumes and incubation times.
- Keep all buffers cold.

Indirect versus Direct Technique

Use the *indirect technique* when a cocktail of biotinylated monoclonal antibodies is used to deplete several cell types simultaneously (use MNC as a starting sample to remove erythrocytes, platelets, and granulocytes), very high depletion efficiency is required, the affinities of biotinylated antibodies are low, the cells express low number of target antigens, or the direct technique gives unsatisfactory purity.

Use the *direct technique* when the affinity of the primary antibody is high, the cells express a high number of target antigens, or to make a larger stock preparation of primary coated Dynabeads® (will generally have the same shelf life as stated on the Dynabeads® vial).

Protocols

Wash Dynabeads®

See Table 1 and 2 for volume recommendations.

1. Resuspend the Dynabeads® in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Dynabeads® to a tube.
3. Add the same volume of Isolation Buffer, or at least 1 mL, and resuspend.
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 Isolation Buffer as the initial volume of Dynabeads® (step 2).

Prepare Sample

- Cells can be directly isolated from any samples such as whole blood, bone marrow, MNC suspensions, or tissue digests. See “General guidelines” for sample preparation
- Prepare a MNC suspension according to “General Guidelines”. Resuspend the cells at 1 × 10^7 cells/mL in Isolation Buffer.

Isolate Cells - Indirect Technique (Labeling Cells with Biotinylated Antibodies)

- See "General Guidelines" for recommendation of when to use the direct vs. indirect cell isolation technique.
- Use approximately 10 µg of primary biotinylated antibody per 10^7 target cells.

This protocol is based on 1 × 10^7 MNC or 1 mL whole blood, but is directly scalable from 1 × 10^7 to 4 × 10^8 cells or 1–40 mL whole blood. When working with lower volumes than 1 × 10^7 cells or 1 mL blood, use the same volumes as for 1 × 10^7 cells or 1 mL blood. When working with larger volumes, scale up all reagent and volumes accordingly, as shown in Table 1.

1. Add ~10 ug primary antibody to 1 mL cell suspension and mix (titrate the antibody amount for your use).
2. Incubate for 10 min at 2°C to 8°C.
3. Wash the cells by adding 2 mL Isolation Buffer and centrifuge at 350 × g for 8 min. Discard the supernatant.
4. Resuspend the cells in Isolation Buffer to 1 × 10^7 MNC per mL (or 1 mL for blood).
5. For *positive isolation or depletion* of one cell type, add 25 µL pre-washed and resuspended Dynabeads®. For *negative isolation* (removal of multiple cell types simultaneously) add 100 µL Dynabeads®.
6. Incubate for 20 min (positive isolation) or 30 min (depletion/negative isolation) at 2°C to 8°C with gentle tilting and rotation.
7. *Optional:* Add 1 mL Isolation Buffer to limit trapping of unbound cells.

8. Place the tube in a magnet for 2 min.
9. *Negative isolation/depletion:* Transfer the supernatant containing the unbound cells to a fresh tube for further experiments.
   or
   Positive isolation: While the tube is still in the magnet, carefully remove and discard the supernatant.
10. Remove the tube from the magnet and add 1 mL Isolation Buffer, pipet 2–3 times (or vortex 2–3 sec) and place the tube in a magnet for 2 min.
11. Repeat steps 9–10 at least twice to wash the cells and obtain high purity.
12. Resuspend the cell pellet in preferred cell medium.

Table 1: Volumes for indirect cell isolation.

Step	Step description	Volumes per 1×10^7 MNC	Volumes per 2×10^8 MNC
	Recommended tube size	5–7 mL	50 mL
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	Primary biotinylated antibody	~10 µg	~200 µg
1	Cell volume (MNC/blood)	1 mL	20 mL
3*	Wash cells (Isolation Buffer)	~2 mL	~40 mL
4	Resuspend cells	1 mL	20 mL
5**(*)	Add Dynabeads® (positive isolation/depletion) Add Dynabeads® (negative isolation)	25 µL 100 µL	500 µL 2 mL
7*	Increase volume (Isolation Buffer)	~1 mL	~ 20 mL
10–12*	**For positive isolation only:** Wash cells (Buffer 1)	3 × ~1 mL	3 × 20 mL

* Adjust the Isolation Buffer volumes to fit to the tube you are using.
** If very high depletion efficiency is required or you are depleting many cells simultaneously, you might have to increase/ optimize the amount of Dynabeads®.
*** When incubating, tilt and rotate so the cells and beads are kept in the bottom of the tube. Do not perform end-over-end mixing if the volume is small relative to the tube size.

Isolate Cells – Direct Technique (Antibody-coating of Dynabeads®)

- See "General Guidelines" for recommendation of when to use the direct vs. indirect cell isolation technique.
- Use 0.5–1.5 µg of primary biotinylated antibody per 25 µL (1×10^7) Dynabeads®. Titrate the primary antibody to optimize the amount used.

This protocol is based on 1×10^7 MNC or 1 mL whole blood, but is directly scalable from 1×10^7 to 4×10^8 cells or 1–40 mL whole blood. When working with lower volumes than 1×10^7 cells or 1 mL blood, use the same volumes as for 1×10^7 cells or 1 mL blood. When working with larger volumes, scale up all reagent and volumes accordingly, as shown in Table 2.

1. Transfer 25 µL pre-washed and resuspended Dynabeads® to a tube.
2. Add ~1 ug antibodies (titrate the antibody amount for your use).
3. Incubate for ≥30 minutes at room temperature with gentle tilting and rotation.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and add 2 mL Isolation Buffer.
6. Repeat steps 4–5 once to remove excess of antibodies.
7. Place the tube in the magnet for 1 min and discard the supernatant.
8. Add to the beads to 1 mL cell sample (blood or 1×10^7 MNC) and resuspend.
9. Incubate for 20 min (positive isolation) or 30 min (depletion) at 2°C to 8°C with gentle tilting and rotation
10. *Optional:* Add 1 mL Isolation Buffer to limit trapping of unbound cells.
11. Place the tube in a magnet for 2 min.
12. *Depletion:* Transfer the supernatant containing the unbound cells to a fresh tube for further experiments.
    or
    Positive isolation: While the tube is still in the magnet, carefully remove and discard the supernatant.
13. Remove the tube from the magnet and add 1 mL Isolation Buffer, pipet 2–3 times (or vortex 2–3 sec) and place the tube in a magnet for 2 min.
14. Repeat steps 12–13 at least twice to wash the cells and to obtain high purity.
15. Resuspend the cell pellet in preferred cell medium.

Table 2: Volumes for direct cell isolation.

Step	Step description	Volumes per 1×10^7 MNC	Volumes per 2×10^8 MNC
	Recommended tube size	5–7 mL	50 mL
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	Dynabeads®	25 µL	500 µL
2	Primary biotinylated antibody	~1 ug	~20 ug
5-6	Wash Dynabeads® (Isolation Buffer)	2 x ~2 mL	2 x ~40 mL
8	Cell volume	1 mL	20 mL
10*	*Optional:* Increase volume (Isolation Buffer)	~1 mL	~8 mL
13-14*	**For positive isolation only:** Wash the cells (Isolation Buffer)	3 × ~1 mL	3× 20 mL

* Adjust the Buffer volumes to fit to the tube you are using.
** If the target cell population is high (e.g. >2.5×10^6 target cells/mL), increase/double the amount of Dynabeads®.
*** When incubating, tilt and rotate so the cells and beads are kept in the bottom of the tube. Do not perform end-over-end mixing if the volume is small relative to the tube size.

Description of Materials

Dynabeads® Biotin Binder are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with 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.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
Dynabeads® FlowComp™ Flexi	11061D
CELLection™ Biotin Binder	11533D
HulaMixer® Sample Mixer	15920D

REF on labels is the symbol for catalog number.

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