Dynabeads™ Biotin Binder

Catalog Numbers 11047, 11048D

Pub. No. MAN1000857 Rev. A

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Invitrogen™ 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.

For positive isolation, discard the supernatant and use the bead-bound cells for downstream applications (e.g. isolation of proteins, nucleic acids, or cell culture). If bead-free cells are required for downstream cellular applications or use in flow cytometry, use the equivalent CELLection™ Biotin Binder Kit, which uses cleavage of a DNA-linker to release the cells from the beads. For the isolation of phagocytic cells, use the Dynabeads™ FlowComp™ Flexi Kit, which can be used at the lower temperatures necessary to reduce phagocytic activity.

Technology overview

Dynabeads™ Biotin Binder are uniform superparamagnetic beads (2.8 µm diameter) coated with recombinant streptavidin. The streptavidin coated onto magnetic beads 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.

Dynabeads™ Biotin Binder contains 4×10^8 beads/mL in phosphate buffered saline (PBS), pH 7.4, with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative.

For this manual protocol, 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. The beads are then mixed with the cell sample in a tube. The beads bind to the target cells during a short incubation, allowing the bead-bound cells to be separated using a magnet.

For depletion: Discard the bead-bound cells and use the remaining bead-free and untouched cells for downstream applications.

For positive isolation: Discard the supernatant and use the bead-bound cells for downstream applications (e.g. isolation of proteins, nucleic acids, or cell culture).

Contents and storage

Item	Cat. No.	Amount	Product capacity		Storage
			Whole blood	MNCs	
Dynabeads™ Biotin Binder	11048D	2 mL	80 mL	~8×10^8 cells	2–8°C Do not freeze.
	11047	5 mL	200 mL	~2×10^9 cells	

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
DynaMag™ magnet	thermofisher.com/magnets
HulaMixer™ Sample Mixer, or equivalent mixer that can tilt and rotate	15920D
Isolation Buffer: Calcium and Magnesium-free PBS supplemented with 0.1% BSA and 2 mM EDTA, pH 7.4.[1]	MLS

[1] BSA can be replaced by human serum albumin (HSA) or fetal calf serum (FCS). EDTA can be replaced by sodium citrate.

Procedural guidelines

- For recommended sample preparation procedures, go to **thermofisher.com/samplepreparation**.
- 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 “Recommended volumes for processing samples” on page 5.
- If very high depletion efficiency is required or you are depleting many cells simultaneously, you might have to increase/ optimize the amount of magnetic beads.
- The choice of primary antibody is the most important factor for successful cell isolation.

  Note: 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 magnetic beads 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.
- Use a mixer that provides tilting and rotation of the tubes to ensure that beads do not settle in the tube.
- This product should not be used with the Dynabeads™ MPC™-1 (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Never use less than the recommended volume of beads.
- Carefully follow the recommended volumes and incubation times.
- When incubating, tilt and rotate the tube to keep the cells and beads at the bottom. Do not perform end-over-end mixing if the volume is small relative to the tube size.
- Keep all buffers cold.

Recommendations for indirect and direct techniques

Use the indirect technique when:

- A cocktail of biotinylated antibodies is used for negative isolation to deplete several cell types simultaneously..
- MNCs are recommended as starting material.
- Very high depletion efficiency is required
- Affinity of biotinylated antibody is low
- Cells express low levels of target antigens
- Direct technique gives unsatisfactory purity

Use the direct technique when:

- Affinity of the primary antibody is high
- Cells express a high level of target antigens
- A larger stock of primary coated beads is prepared

  Note: Primary coated Dynabeads™ will generally have the same shelf life as stated on the vial.

Prepare magnetic beads

For recommended volumes, see “Recommended volumes for processing samples” on page 5.

1. Vortex the vial of Dynabeads™ magnetic beads for at least 30 seconds, or tilt and rotate the vial for 5 minutes.
2. Transfer the required volume of beads to an appropriately-sized tube, then add the same volume of Isolation Buffer (at least 1 mL) to the tube and resuspend the beads.
3. Place the tube on a magnet for 1 minute, then discard the supernatant.
4. Remove the tube from the magnet, then resuspend the washed beads in the same volume of Isolation Buffer as the initial volume of magnetic beads used (see Step 2).

Prepare samples

- For recommended sample preparation procedures, go to **thermofisher.com/samplepreparation**.
- Cells can be directly isolated from any sample such as whole blood, bone marrow, MNC suspensions or tissue digests.
- Prepare MNCs to 1×10^7 cells/mL in Isolation Buffer.
- For recommendations of when to use the direct versus indirect isolation technique, see “Recommended volumes for processing samples” on page 5.

Isolate cells with indirect or direct techniques

These protocols are based on 1×10^7 MNCs or 1 mL whole blood, but are directly scalable from 1×10^7 to 4×10^8 MNCs or 1–40 mL whole blood. When working with volumes less than 1×10^7 cells or 1 mL blood, use the same volumes as for 1×10^7 cells or 1 mL blood. For larger volumes, scale up all volumes accordingly. When working with larger volumes, scale up all reagent and volumes accordingly. See “Recommended volumes for processing samples” on page 5.

Isolate cells using the indirect technique

1. Add ~10 µg of primary antibody to 1 mL of cell suspension, then mix.

   Note: Use approximately 10 µg of primary antibody (biotinylated) per 10^7 target cells. Titrate the primary antibody to optimize the amount used.
2. Incubate for 10 minutes at 2–8°C.
3. Wash the cells by adding 2 mL of Isolation Buffer, then centrifuge at 350 × *g* for 8 minutes. Discard the supernatant.
4. Resuspend the cells in Isolation Buffer to 1×10^7 MNC/mL (or 1 mL for blood).

5. Add Dynabeads™:
   - For positive isolation or depletion of one cell type, add 25 µL of the prepared beads.
   - For negative isolation (removal of multiple cell types simultaneously), add 100 µL of the prepared beads.
6. Incubate for 20 minutes (positive isolation) or 30 minutes (depletion/negative isolation) at 2–8°C with gentle tilting and rotation.
7. *Optional*: Add 1 mL Isolation Buffer to limit trapping of unbound cells.
8. Place the tube on the magnet for 2 minutes.
9. Deplete or isolate cells:
   - For depletion or negative isolation, transfer the supernatant containing the unbound cells to a fresh tube for further experiments.
   - For positive isolation, while the tube is still on the magnet, carefully remove, then discard the supernatant.
10. Remove the tube from the magnet then add 1 mL of Isolation Buffer. Pipet up and down 2–3 times (or vortex 2–3 seconds).
11. Place the tube on the magnet for 2 minutes. While the tube is still on the magnet, carefully remove, then discard the supernatant.
12. Repeat step 10–step 11, at least twice to wash the cells.

    Note: These steps are critical to obtain a high purity of isolated cells.
13. Resuspend the bead-bound cells in preferred cell medium or lysis buffer for downstream molecular analysis.

Isolate cells using the direct technique

1. Transfer 25 µL of the prepared magnetic beads to a tube.
2. Add ~1 µg of antibodies (titrate the antibody amount for your use).

   Note: Use 0.5–1.5 µg of primary antibody (biotinylated) per 25 µL (1×10^7) of magnetic beads. Titrate the primary antibody to optimize the amount used.
3. Incubate for ≥30 minutes at room temperature with gentle tilting and rotation.
4. Place the tube on the magnet for 1 minute, then discard the supernatant.
5. Remove the tube from the magnet, then add 2 mL of Isolation Buffer.
6. Repeat step 4–step 5, once to remove excess of antibodies.
7. Place the tube in the magnet for 1 minute, then discard the supernatant.
8. Remove the tube from the magnet, then add 1 mL of cell sample ($1x10^7$ MNCs or blood) to the bead pellet. Resuspend the bead pellet.
9. Incubate for 20 minutes (positive isolation) or 30 minutes (depletion) at 2–8°C with gentle tilting and rotation.
10. *Optional*: Add 1 mL of Isolation Buffer to limit trapping of unbound cells.
11. Place the tube in a magnet for 2 minutes.
12. Deplete or isolate cells:
    - For depletion, transfer the supernatant containing the unbound cells to a fresh tube for further experiments.
    - For positive isolation, while the tube is still in the magnet, carefully remove, then discard the supernatant.
13. Remove the tube from the magnet, then add 1 mL of Isolation Buffer. Pipet up and down 2–3 times (or vortex 2–3 seconds).
14. Place the tube on the magnet for 2 minutes. While the tube is still in the magnet, carefully remove, then discard the supernatant.

15. Repeat step 13–step 14, at least twice to wash the cells.

    Note: These steps are critical to obtain a high purity of isolated cells.

16. Resuspend the bead-bound cells in preferred cell medium or lysis buffer for downstream molecular analysis.

Recommended volumes for processing samples

Table 1 Volumes for indirect cell isolation

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

[1] Adjust the Isolation Buffer volumes to fit the tube you are using.
[2] If very high depletion efficiency is required or you are depleting many cells simultaneously, you might need to increase the amount of magnetic beads.

Table 2 Volumes for direct cell isolation

Step	Step description	Volume	
		Volumes per 1 × 10^7 MNC	Volumes per 2 × 10^8 MNC
–	Recommended tube size	5–7 mL	50 mL
–	Recommended magnet	DynaMag™-5 Magnet	DynaMag™-50 Magnet
1[1,2]	Beads	~25 µL	~500 µL
2[1]	Primary antibody (biotinylated)y	~1 µg	~20 µg
4[3]	Isolation Buffer	1 mL	20 mL
5–6	Wash beads (Isolation Buffer)	2 × ~2 mL	2 × ~40 mL
9	Cell volume	1 mL	20 mL
11[3]	Optional: Increase volume (Isolation Buffer)	~1 mL	~8 mL
14–16[3]	For positive isolation only: Wash cells (Isolation Buffer)	3 × ~1 mL	3 × ~20 mL

[1] If the target cell population is high (e.g. >2.5 × 10^6 target cells/mL), increase the magnetic bead volume up to double the amount of beads and antibody.
[2] If very high depletion efficiency is required or you are depleting many cells simultaneously, you might need to increase the amount of magnetic beads.
[3] Adjust the Buffer volumes to fit the tube you are using.

Related products

Product	Cat. No.
CELLection™ Biotin Binder Kit	11047
Dynabeads™ FlowComp™ Flexi Kit	11061D

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN1000857 A

Revision	Date	Description
A	13 January 2025	• Initial release with new publication number. Supersedes SPEC number SPEC-05724, Rev. 004. • The version numbering was changed from a numerical format to a letter-based format in accordance with internal document control procedures. • New catalog number added for 2 mL quantity of Dynabeads™ Biotin Binder.