Dynabeads™ CD3

Catalog Numbers 11151D, 11152D

Pub. No. MAN1000848 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**.

This guide describes manual isolation or depletion of human CD3+ T cells. For automated instructions, see *Automated Dynabeads™ - Based Cell Capture using the KingFisher™ Apex or KingFisher™ Flex Purification Systems User Guide* (Pub. No. MAN1000759).

Product description

The Invitrogen™ Dynabeads™ CD3 contains superparamagnetic beads that enable isolation or depletion of human CD3+ T cells directly from whole blood, buffy coat, or mononuclear cells (MNCs).

For rapid and consistent results in protein or gene expression analysis, lyse the CD3+ T cells while they are still bound to the beads, then immediately proceed with downstream molecular analysis. For positive isolation intended for functional studies, cell activation/expansion, or flow cytometry analysis, the cells must be released after isolation. In such cases, Dynabeads™ FlowComp™ Human CD3 Kit is recommended for obtaining bead-free cells.

Technology overview

Dynabeads™ CD3 are uniform superparamagnetic beads (4.5 µm diameter) that are coated with a primary mouse monoclonal antibody directed against the CD3 membrane antigen mainly expressed on human mature T cells. The primary CD3 antibody is coupled to the beads via a secondary rat anti-mouse IgG antibody.

Dynabeads™ CD3 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 beads are mixed with the cell sample in a tube. The beads bind to the target cells during a short incubation, and then the bead-bound cells are separated by a magnet.

For depletion: Discard the bead-bound cells and use the remaining, untouched cells for any downstream application.

For positive isolation: Discard the supernatant and use the bead-bound cells for downstream molecular applications.

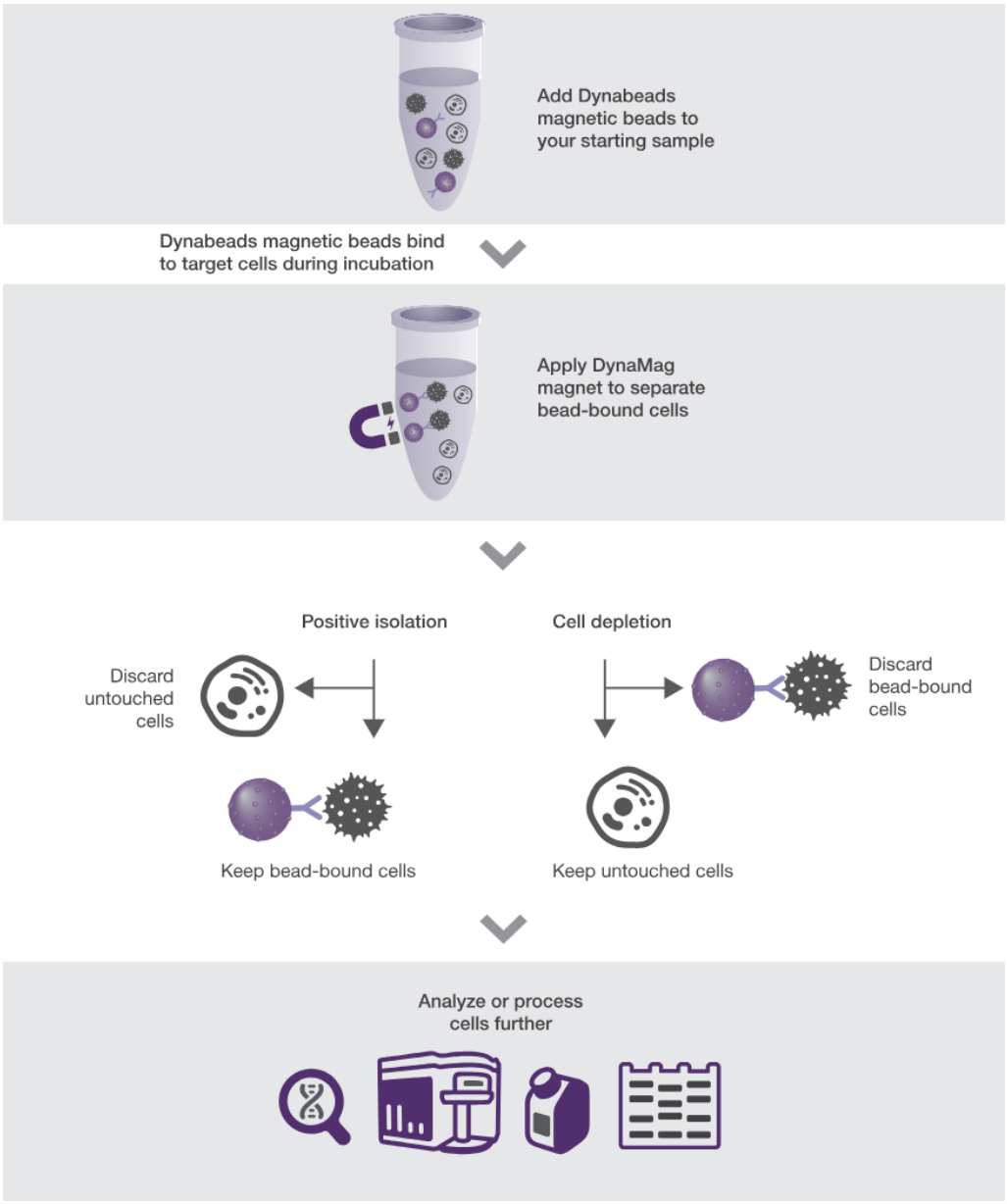


Figure 1 Workflow for manual method

Contents and storage

Item	Cat. No.	Amount	Product capacity		Storage
			Whole blood	MNCs	
Dynabeads™ CD3	11152D	2 mL	80 mL	~8 × 10^8 cells	2–8°C Do not freeze.
	11151D	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 high cell-depletion efficiency is required, increase the beads volume up to double the recommended amount.
- Cells can be directly isolated from any sample such as whole blood, bone marrow, MNC suspensions, or tissue digests. Whole blood and buffy coat need to be washed before isolation to remove interfering soluble factors.
- 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.
- Carefully follow the recommended pipetting volumes and incubation times.
- Keep all buffers cold.

Prepare magnetic beads

For recommended volumes, see “Recommended volumes for processing samples” on page 4.

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 appropriate volume of beads to an appropriately-sized tube, then add the same volume of Isolation Buffer (at least 1 mL) to the tube to 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 beads used (see step 2).

Prepare samples

Prepare samples as described.

Table 1 Sample preparation procedures

Sample type	Action
Whole blood Buffy coat	**Note:** Wash the sample to remove any interfering soluble factors. Buffy coat has an 8–10 times greater concentration of leukocytes than whole blood and should be diluted before use. 1. Dilute the whole blood or buffy coat 1:2 with Isolation Buffer — Combine 1 part sample with 2 parts Isolation Buffer. 2. Centrifuge at 600 × *g* for 10 minutes at 2–8°C. 3. Discard the upper layer (plasma fraction). 4. Resuspend whole blood to the original volume in Isolation Buffer and buffy coat to 2X the original volume in Isolation Buffer before adding the magnetic beads.
MNCss	1. Prepare MNCs as described. Go to thermofisher.com/samplepreparation. 2. Resuspend MNCs in Isolation Buffer at a concentration of 1×10^7 cells/mL.

Deplete or positively isolate cells

The protocol is based on 1 mL (1×10^7) MNCs or 1 mL washed whole blood/ washed and diluted buffy coat as starting sample, but is scalable from 1×10^7 – 5×10^8 MNCs (1–50 mL). When working with volumes less than 1 mL, use the same volumes as indicated for 1 mL. For larger volumes, scale up all volumes. See "Recommended volumes for processing samples" on page 4.

1. Transfer 1 mL of cells (1×10^7) to a tube, then add 25 µL of the prepared beads.
2. Incubate for 20 minutes (positive isolation) or 30 minutes (depletion) at 2–8°C with gentle tilting and rotation.
3. Place the tube on the magnet for 2 minutes.
4. Deplete or isolate cells:
   - For depletion, transfer the supernatant to a new tube for further use, then discard the beads.
   - For positive isolation, carefully remove, then discard the supernatant while the tube is still on the magnet.
5. 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).
6. Place the tube on the magnet for 2 minutes. While the tube is still on the magnet, carefully remove, then discard the supernatant.
7. Repeat step 5–step 6 at least once to wash the bead-bound CD3+ T cells.

   Note: This step is critical to obtain a high purity of isolated cells.
8. Resuspend the bead-bound cells in the preferred cell medium or lysis buffer for downstream molecular analysis.

Keep the cells at 2–8°C until use in downstream applications.

Recommended volumes for processing samples

This protocol is scalable from 1×10^7 to 5×10^8 cells.

Table 2 Volumes for isolation or depletion of human CD3+ T cells

Step	Step description	Volume	
		Small scale (1X)	Large scale (10X)
–	Recommended tube size	5 mL	15 mL
–	Recommended magnet	DynaMag™-5 Magnet	DynaMag™-15 Magnet
1[1]	Sample volume (MNCs/blood/buffy)	1 mL	10 mL
1	Bead volume	25 µL	250 µL
5–6	For positive isolation only: Wash cells (Isolation Buffer)	3 × ~1 mL	3 × ~10 mL

[1] 1×10^7 MNCs/mL

Related products

Product	Cat. No.
Dynabeads™ Human T-Activator CD3/CD28	11161D
Dynabeads™ FlowComp™ Human CD3 Kit	11365D

Limited product warranty

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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. MAN1000848 A

Revision	Date	Description
A	10 January 2025	• Initial release with new publication number. Supersedes SPEC number SPEC-05870, Rev. 003. • 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™ CD3.

The information in this guide is subject to change without notice.

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