Dynabeads™ CD4 Positive Isolation Kit

Catalog Number 11331D

Pub. No. MAN1000061 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™ CD4 Positive Isolation Kit contains superparamagnetic beads that enable gentle isolation of high-purity CD4+ T cells from whole blood, bone marrow, buffy coat, mononuclear cells (MNCs), or tissue digests. The cells are released from the beads using the provided DETACHaBEAD™ reagent. Isolated CD4+ T cells are bead- and antibody-free, phenotypically unaltered, and appropriate for any downstream application, including flow cytometry, functional studies, and cell culture.

Technology overview

Dynabeads™ CD4 are uniform superparamagnetic polystyrene beads (4.5 µm diameter) that are coated with a primary monoclonal antibody directed against the CD4 membrane antigen, which is predominantly expressed on the helper/inducer subset of human T cells. DETACHaBEAD™ CD4 is a polyclonal anti-Fab antibody that is specific for the CD4 antibody on Dynabeads™ CD4.

Dynabeads™ CD4 is mixed with the sample in a tube. The beads bind to the target cells during a short incubation. The bead-bound cells are washed, then isolated CD4+ cells are gently released from the beads by addition of the DETACHaBEAD™ reagent.

Contents and storage

The kit can process up to 400 mL of whole blood or up to 2×10^9 MNCs.

Table 1 Dynabeads™ CD4 Positive Isolation Kit (Cat. No. 11331D)

Item	Amount	Storage
Dynabeads™ CD4[1]	5 mL	2–8°C Do not freeze.
DETACHaBEAD™ CD4[2,3]	2 mL	

[1] Dynabeads™ CD4 contains 4×10^8 beads/mL in phosphate buffered saline (PBS), with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative.
[2] DETACHaBEAD™ CD4 contains a polyclonal anti-Fab antibody in 0.15 M PBS.
[3] Due to the high protein concentration of the DETACHaBEAD™ reagent, some precipitates may form in the vial. This is not a cause for concern and does not negatively affect performance.

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
Buffer 1: PBS (without Ca^{2+} and Mg^{2+}) with 0.1% BSA and 2 mM EDTA, pH 7.4[1]	MLS
Buffer 2: RPMI 1640 with 1% FCS	MLS

[1] BSA can be replaced by human serum albumin (HSA) or fetal calf serum (FCS). EDTA can be replaced by sodium citrate. PBS containing Ca^{2+} or Mg^{2+} is not recommended.

Procedural guidelines

- For recommended sample preparation procedures, go to **thermofisher.com/samplepreparation**.
- This guide describes the procedure for processing 1 mL of MNCs (1×10^7 cells) or 1 mL of washed whole blood or buffy coat, however, volumes can be scaled for processing up to 50 mL of MNCs (5×10^8 cells). For cell counts $<1 \times 10^7$ cells, use the same volumes indicated for 1×10^7 cells. For cell counts $>1 \times 10^7$ cells, scale the volumes proportionally.
- Use an appropriately-sized tube and magnet, according to the following table.

Table 2 Recommended tube and magnet sizes

Sample volume	Recommended tube	Recommended magnet
1 mL (small scale)	5-mL tube	DynaMag™-5 Magnet
10 mL (medium scale)	15-mL tube	DynaMag™-15 Magnet

- Use a mixer that can tilt and rotate the tubes to maintain the Dynabeads™ beads in suspension.
- Avoid air bubbles (foaming) during pipetting.
- Carefully follow the recommended pipetting volumes and incubation times.
- Keep all buffers cold.

Recommended volumes

Table 3 Recommended volumes for processing prepared whole blood, buffy coat, and MNCs

Step	Reagent	Volume	
		Small scale	Medium scale
Add sample to tube	Cell sample	1 mL	10 mL
Combine sample with Dynabeads™ CD4	Dynabeads™ CD4	For buffy coat and MNCs—Use 25 µL. For blood—Use 12.5 µL.	For buffy coat and MNCs—Use 250 µL. For blood—Use 125 µL.
Wash bead-bound cells	Buffer 1	3 × 1 mL	3 × 10 mL
Resuspend bead-bound cells	Buffer 2	100 µL[1]	1 mL[1]
Release cells from Dynabeads™ CD4	DETACHaBEAD™ reagent	10 µL	100 µL
Collect bead-free cells	Buffer 2	3 × 500 µL	3 × 5 mL
Wash bead-free cells	Buffer 2	Total of 4 mL[2]	Total of 10 mL[2]

[1] Transfer the sample to an appropriately-sized tube (for example, a microcentrifuge tube (small scale) or 5-mL tube (medium scale)).
[2] The wash volume is related to the original tube size. Do not use less than the recommended volume.

Wash Dynabeads™ magnetic beads

For recommended volumes, see “Recommended volumes” on page 2.

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 Dynabeads™ magnetic beads to an appropriately-sized tube, then add the same volume of Buffer 1 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 Buffer 1 as the initial volume of Dynabeads™ magnetic beads used.

Prepare samples

Prepare samples as described.

Table 4 Sample preparation procedures

Sample type	Action
Whole blood Buffy coat	**Note:** Buffy coat has an 8–10 times greater concentration of leukocytes than whole blood. 1. Wash the sample to remove any interfering soluble factors. 2. Dilute 1:2 with Buffer 1—Combine 1 part sample with 2 parts Buffer 1. 3. Centrifuge at 600 × *g* for 10 minutes at 2–8°C. Decelerate slowly. 4. Discard the upper layer (plasma fraction). 5. Resuspend the sample in Buffer 1. • **For blood—**Add the same volume of Buffer 1 as the initial volume of blood used. • **For buffy coat—**Add double the volume of Buffer 1 as the initial volume of buffy coat used.
MNCs	1. Prepare MNCs as described. Go to thermofisher.com/samplepreparation. 2. Resuspend MNCs in Buffer 1 at a concentration of 1×10^7 cells/mL.

Isolate CD4+ T cells

The following procedure is optimized for isolating CD4+ T cells from 1 mL of prepared MNCs (1×10^7 cells), or 1 mL of prepared whole blood or buffy coat. Scale volumes proportionally to process up to 50 mL of MNCs (5×10^8 cells). See "Recommended volumes" on page 2.

1. Add the appropriate volume of Dynabeads™ magnetic beads to the prepared sample. For example, add 25 µL of beads to 1 mL of prepared MNCs.
2. Incubate for 20 minutes at 2–8°C with gentle tilting and rotation.
3. Place the tube on a magnet for 2 minutes.
4. Keeping the tube on the magnet, carefully remove then discard the supernatant.
5. Remove the tube from the magnet, then add 1 mL of Buffer 1. Pipet 2–3 times (or vortex 2–3 seconds) to mix, then place the tube back on the magnet for 2 minutes.
6. Repeat step 4 and step 5 twice to wash the bead-bound CD4+ T cells.

   IMPORTANT! Follow the wash steps as described to ensure isolation of high-purity CD4+ T cells.

7. Resuspend the pellet in 100 µL of Buffer 2.

Release CD4+ T cells

1. Add 10 µL of DETACHaBEAD™ reagent to the tube.
2. Incubate for 45 minutes at room temperature with gentle tilting and rotation.
3. Place the tube on a magnet for 1 minute.
4. Keeping the tube on the magnet, transfer the supernatant containing the released (bead-free) cells to a new tube.

   Note: To collect any remaining cells, wash the beads 2–3 times in 500 µL of Buffer 2, then collect the supernatant after separation on a magnet.
5. Wash the cells thoroughly to remove the DETACHaBEAD™ reagent—Bring the total volume of the cell suspension to 4 mL with Buffer 2, then centrifuge at 400 × *g* for 6 minutes.
6. Remove, then discard all visible liquid without disturbing the pellet.
7. Resuspend the cells in Buffer 2 or other media that is appropriate for your downstream application.

The isolated CD4+ T cells are ready for use in a wide range of downstream applications.

Related products

Item	Source
DynaMag™-5 Magnet	12303D
DynaMag™-15 Magnet	12301D
DynaMag™-50 Magnet	12302D
Dynabeads™ CD8 Positive Isolation Kit	11333D

Limited product warranty

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

Revision history: Pub. No. MAN1000061 A

Revision	Date	Description
A	23 May 2024	• The legacy document (Rev.005 November 2011) was converted to the current document template, with associated updates to the limited license information, warranty, trademarks, and logos. • The version format was changed in conformance with internal document control procedures. • Minor edits were incorporated for clarity and consistency with related documents.