

# Dynabeads® CD4 Positive Isolation Kit

Catalog no. 11331D

Store at 2 to 8°C

Rev. Date: November 2011 (Rev. 005)

## Kit Contents

Kit contents	Volume
Dynabeads® CD4	5 mL
DETAHaBEAD® CD4	2 mL

### Kit capacity

Whole blood: 400 mL

MNC:  $\sim 2 \times 10^9$

Dynabeads® CD4 contains  $4 \times 10^8$  beads/mL in phosphate buffered saline (PBS), with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative. DETAHaBEAD® CD 4 contains a polyclonal anti-Fab antibody in 0.15 M PBS.

## Product Description

Positively isolate a high yield and purity of CD4<sup>+</sup> T cells from whole blood, buffy coat, mononuclear cells (MNCs), or bone marrow and then remove the beads using the supplied DETAHaBEAD®. Isolated cells are bead and antibody-free, phenotypically unaltered and suitable for any downstream application, including flow cytometry, functional studies and cell culture.

Dynabeads® are mixed with the 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. Wash the positively isolated cells and add DETAHaBEAD® to gently release the cells from the beads.

## Required Materials

- Magnet (DynaMag™): See [www.lifetechnologies.com/magnets](http://www.lifetechnologies.com/magnets) for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Buffer 1: PBS (Ca<sup>2+</sup> and Mg<sup>2+</sup> free) with 0.1% 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. PBS containing Ca<sup>2+</sup> or Mg<sup>2+</sup> is not recommended.
- Buffer 2: RPMI 1640/1% FCS.

## General Guidelines

- Visit [www.lifetechnologies.com/samplepreparation](http://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 in the tube.
- This product should not be used with MPC™-1 (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Carefully follow the recommended pipetting volumes and incubation times.
- Keep all buffers cold.

## Protocol

### Wash Dynabeads®

See Table 1 for volume calculations.

1. Resuspend the Dynabeads® in the vial (vortex >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 Buffer 1, 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 Buffer 1 as the initial volume of Dynabeads®.

### Prepare Whole Blood and Buffy Coat

Wash the blood/buffy coat to remove interfering soluble factors. Note that buffy coat has 8–10 times higher concentration of leucocytes than whole blood.

1. Dilute the whole blood or buffy coat in Buffer 1 (1 part blood/buffy coat to 2 parts Buffer 1).
2. Centrifuge at  $600 \times g$  for 10 min at 2°C to 8°C. Allow to decelerate slowly.
3. Discard the plasma fraction/upper layer. Resuspend blood to the original volume with Buffer 1. Resuspend Buffy coat to double the original volume with Buffer 1.

### Prepare MNC

- Prepare MNC according to "General Guidelines".
- Resuspend the MNC to  $1 \times 10^7$  cells/mL in Buffer 1.

### Isolate CD4<sup>+</sup> T Cells

The isolation and release protocol is based on 1 mL MNC ( $1 \times 10^7$  cells), or 1 mL washed whole blood or buffy coat as starting sample, but is scalable from  $1 \times 10^7$  –  $5 \times 10^8$  cells according to Table 1.

1. Add the appropriate volume of Dynabeads® to the prepared sample according to Table 1 (e.g. 25  $\mu$ L beads/mL MNC).
2. Incubate for 20 min at 2°C to 8°C with gentle tilting and rotation.
3. Place the tube in a magnet for 2 min.
4. While the tube is still in the magnet, carefully remove and discard the supernatant.
5. Remove the tube from the magnet and add 1 mL Buffer 1, pipet 2–3 times (or vortex 2–3 seconds) and place the tube in a magnet for 2 min.
6. Repeat steps 4–5 twice to wash the bead-bound CD4<sup>+</sup> T cells. These steps are critical to obtain a high purity of isolated cells.
7. Resuspend the cell pellet in 100  $\mu$ L Buffer 2.

## Release CD4<sup>+</sup> T Cells

8. Add 10  $\mu$ L DETACHaBEAD<sup>®</sup>.
9. Incubate for 45 min at room temperature with gentle mixing.
10. Place the tube in a magnet for 1 min.
11. Transfer the supernatant containing released cells to a fresh tube.  
To obtain residual cells, wash the beads 2–3 times in 500  $\mu$ L Buffer 2 and collect the supernatant.
12. Wash detached cells thoroughly by resuspending the cells to a total volume of 4 mL Buffer 2 and centrifuge for 6 min at 400  $\times$  g to remove DETACHaBEAD<sup>®</sup>. Resuspend the cells in Buffer 2 or other media and use in downstream application.

The isolated cells are pure, viable, and are free from antibody or beads bound to the surface.

Table 1: Volumes for human CD4<sup>+</sup> T cell isolation from PBMC, washed/diluted buffy coat or washed whole blood. The protocol is scalable from 1 mL ( $10^7$  MNC) to 50 mL ( $5 \times 10^8$  MNC). For lower cell numbers than  $10^7$ , use the same volumes as indicated below. For higher cell numbers than  $10^7$ , scale up the volumes accordingly.

Step	Step description	Small scale	Medium scale
	Recommended tube size	5 mL tube	15 mL tube
	Recommended magnet	DynaMag™-5	DynaMag™-15
1	Cell sample	1 mL	10 mL
1	Dynabeads <sup>®</sup> CD4	25 $\mu$ L 12.5 $\mu$ L (for blood)	250 $\mu$ L 125 $\mu$ L (for blood)
4–5	Wash cells (Buffer 1)	1 mL $\times$ 3	10 mL $\times$ 3
7*	Resuspend cells (Buffer 2)	100 $\mu$ L	1 mL
8	Release cells (DETACHaBEAD <sup>®</sup> )	10 $\mu$ L	100 $\mu$ L
11	Collect residual cells (Buffer 2)	500 $\mu$ L $\times$ 3	5 mL $\times$ 3
12**	Wash cells (Buffer 2)	Total of 4 mL	Total of 10 mL

\* Transfer the sample to a smaller tube that is more appropriate to the volume (e.g. micro-centrifuge and 5 mL tube, respectively).

\*\* Wash volume is related to the original tube size. It is not recommended to wash in a smaller volume.

## Description of Materials

Dynabeads<sup>®</sup> CD4 are uniform, superparamagnetic polystyrene beads (4.5  $\mu$ m diameter) coated with a primary monoclonal antibody specific for the CD4 membrane antigen predominantly expressed on the helper/inducer subset of human T cells. DETACHaBEAD<sup>®</sup> CD4 is a polyclonal anti-Fab antibody specific for the CD4 antibody on the Dynabeads<sup>®</sup>.

## Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer <sup>®</sup> Sample Mixer	15920D

**REF** on labels is the symbol for catalog number.

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