invitrogen
by life technologies

Dynabeads® CD8 Positive Isolation Kit

Catalog no. 11333D **Store at 2 to 8°C**

Rev. Date: November 2011 (Rev. 005)

Kit Contents

Kit contents	Volume
Dynabeads® CD8	5 mL
DETACHaBEAD® CD8	2 mL

Kit capacity
Whole blood: 400 mL
MNC: ~2 × 10^9

Dynabeads® CD8 contains 4 × 10^8 beads/mL in phosphate buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative. DETACHaBEAD® CD8 contains a polyclonal anti-Fab antibody in 0.15 M PBS.

Product Description

Positively isolate a high yield and purity of $CD8^+$ T cells from whole blood, buffy coat, mononuclear cells (MNCs), or bone marrow and then remove the beads using the supplied DETACHaBEAD®. 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 DETACHaBEAD® to gently release the cells from the beads.

Required Materials

- Magnet (DynaMag™): See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Buffer 1: PBS (Ca^{2+} and Mg^{2+} 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^{2+} or Mg^{2+} is not recommended.
- Buffer 2: RPMI 1640/1% FCS.

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 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 × 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 × 10^7 cells/mL in Buffer 1.

Isolate $CD8^+$ T Cells

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

1. Add the appropriate volume of Dynabeads® to the prepared sample according to Table 1.
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 $CD8^+$ T cells. These steps are critical to obtain a high purity of isolated cells.
7. Resuspend the cell pellet in 100 µL Buffer 2.

Release CD8+ Cells

8. Add 10 µL DETACHaBEAD®.
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 µL 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 × g to remove DETACHaBEAD®. 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 CD8+ T cell isolation from MNC, washed/diluted buffy coat or washed whole blood. The protocol is scalable from 1 mL (10^7 MNC) to 50 mL (5×10^8 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® CD8	25 µL 12.5 µL (for blood)	250 µL 125 µL (for blood)
4–5	Wash cells (Buffer 1)	1 mL × 3	10 mL × 3
7*	Resuspend cells (Buffer 2)	100 µL	1 mL
8	Release cells (DETACHaBEAD®)	10 µL	100 µL
11	Collect residual cells (Buffer 2)	500 µL × 3	5 mL × 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® CD8 are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a primary monoclonal antibody directed against the CD8 membrane antigen on human T cells. DETACHaBEAD® CD8 is a polyclonal anti-Fab antibody specific for the CD8 antibody on the Dynabeads®.

Related Products

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

REF on labels is the symbol for catalog number.

Limited Use Label License

The purchase of this product conveys to the purchaser the limited, nontransferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Manufactured by Life Technologies AS, Norway. Life Technologies AS complies with the Quality System Standards ISO 9001:2008 and ISO 13485:2003.

SPEC-05754

For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

life technologies™