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Dynabeads® Mouse CD4 (L3T4)

Catalog no. 11445D **Store at 2˚C to 8˚C**

Rev. Date: March 2012 (Rev. 003)

Product Contents

Product contents	Volume
Dynabeads® Mouse CD4 (L3T4)	5 mL

Product capacity
~2 × 10^9 cells

Dynabeads® Mouse CD4 (L3T4) contains 4 × 10^8 beads/mL in phosphate buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative.
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

This product is intended for positive isolation or depletion of murine $CD4^+$ T cells directly from spleen, lymph node cell suspensions, or other samples containing $CD4^+$ T cells. The Dynabeads® are mixed with the cell sample in a tube and will bind to the target cells during a short incubation. The bead-bound cells are separated by a magnet.

Depletion – Discard the bead-bound cells and use the remaining, untouched cells for any application.

Positive isolation – Discard the supernatant and use the bead-bound cells for downstream applications. The cells can be released from the Dynabeads® using DETACHaBEAD® Mouse CD4 (not supplied). Isolated cells are bead and antibody-free, phenotypically unaltered and ideal for any downstream application.

Downstream Applications

For rapid and consistent results in protein or gene expression analysis, lyse the $CD4^+$ T cells while still attached to the beads and directly process for further molecular analysis. For positive isolation for functional studies, cell activation/expansion, or for flow cytometer analysis, the cells need to be released after isolation. For this, we recommend using DETACHaBEAD® Mouse CD4 in combination with this product (bead-and antibody-free cells). Alternatively, use Dynabeads® FlowComp™ Mouse CD4 (bead-free cells). See "Related Products" for recommendation of products for activation/expansion of T cells.

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes, e.g. HulaMixer® Sample Mixer.
- Isolation Buffer: Ca^{2+} and Mg^{2+} free PBS pH 7.4 with 0.1% BSA and 2 mM EDTA. **Note:** BSA can be replaced by human serum albumin (HSA) or 2% fetal bovine serum (FBS)/ fetal calf serum (FCS).
- Recommended culture media: RPMI 1640 or DMEM with 10% 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.
- Keep the temperature at 2°C to 8°C when incubating Dynabeads® and cells, to minimize phagocytic activity and other metabolic processes.
- Follow the recommended volumes and incubation times.
- Avoid air bubbles (foaming) during pipetting.

Protocol

Wash Dynabeads®

See Table 1 for volume recommendations.

1. Resuspend the Dynabeads® in the vial (i.e. vortex for >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 Isolation Buffer, 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 Isolation Buffer as the initial volume transferred of Dynabeads® (step 2).

Prepare Sample

- Prepare a single cell suspension from lymphoid organs (e.g. lymph nodes or spleen) according to “General Guidelines”.
- Resuspend the cells at 1 × 10^7 cells/mL in Isolation Buffer.

Positively Isolate or Deplete Mouse $CD4^+$ T Cells

This protocol is based on 1 × 10^7 cells, but is directly scalable from 1 × 10^7 to 5 × 10^8 cells. When working with fewer cells than 1 × 10^7, use the same volumes as indicated for 1 × 10^7. When working with higher cell numbers, scale up all volumes accordingly, as shown in Table 1.

1. Transfer 1 mL cells (1 × 10^7) to a tube and add 25 µL pre-washed and re-suspended Dynabeads®.
2. Incubate for 20 min (positive isolation) or 30 min (depletion) at 2°C to 8°C with gentle tilting and rotation.
3. Place the tube in a magnet for 2 min.
4. For *depletion;* transfer supernatant to a new tube for further use and discard the beads.
   or
   For *positive isolation;* 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 Isolation Buffer, pipet 2–3 times (or vortex 2–3 sec) and place the tube in a magnet for 2 min. While the tube is still in the magnet, carefully remove and discard the supernatant.
6. Repeat step 5 at least once to wash the bead-bound $CD4^+$ T cells. This step is critical to obtain a high purity of isolated cells.
7. Resuspend the cell pellet in preferred cell medium.

Keep the cells on 2°C to 8°C until further use in downstream applications.

Table 1: Volumes for isolation/depletion of mouse CD4+ cells. This protocol is scalable from 1 × 10^7 to 5 × 10^8 cells.

Step	Step description	Volumes per 1 × 10^7 cells	Volumes per 1 × 10^8 cells
	Recommended tube size	5 mL	15 mL
	Recommended magnet	DynaMag™-5	DynaMag™-15
1	Cell volume	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

* If very high cell depletion-efficiency is required, increase the Dynabeads® volume up to double the recommended amount.

Description of Materials

Dynabeads® Mouse CD4 (L3T4) are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a monoclonal rat anti-mouse antibody specific for the L3T4 membrane antigen expressed on thymocytes and the T helper subpopulation of mature T cells of all common mouse strains.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D
Dynabeads® Mouse T-Activator CD3/CD28	11456D
Dynabeads® Flowcomp™ Mouse CD4	11461D
DETACHaBEAD® Mouse CD4	12406D

REF on labels is the symbol for catalog number.

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