

DETACHaBEAD® Mouse CD4

Catalog no. 12406D

Store at 2°C to 8°C

Rev. Date: March 2012 (Rev. 007)

Product Contents

Product contents	Volume
DETACHaBEAD® Mouse CD4	5 mL

Product capacity

1 mL DETACHaBEAD® Mouse CD4 is sufficient to release cells from 2.5 mL Dynabeads® Mouse CD4 (L3T4).

DETACHaBEAD® Mouse CD4 is polyclonal antibodies, supplied in 0.15 M phosphate buffered saline (PBS) pH 7.4.

Product Description

DETACHaBEAD® Mouse CD4 is intended for release of mouse CD4⁺ cells from Dynabeads® after positive isolation with Dynabeads® Mouse CD4 (L3T4) (not supplied). The released mouse CD4⁺ cells are pure, viable, not activated by the release-step and have no Dynabeads® or primary antibody bound to the surface. Release of Dynabeads® from the cells using DETACHaBEAD® provides a fast and reliable method of positive isolation of mouse CD4⁺ T cells isolated from mouse spleen, thymus or lymph nodes.

After positive isolation, the DETACHaBEAD® Mouse CD4 is added to the bead-bound cells to release the Dynabeads® and primary antibody from the mouse CD4⁺ T cells. Following a short incubation period, the released Dynabeads® are removed by magnetic separation and the positively selected cells collected.

Downstream Applications

The isolated cells are pure, viable, and are free from antibody bound to the surface. The isolated cells can be used for any research application, functional studies and flow cytometry. Cells positively isolated and released in this way have been shown to respond in a normal manner when stimulated with interleukin-2 or interferon-gamma. Alternatively, if you want both cell isolation and bead release in one kit, use Dynabeads® FlowComp™ Mouse CD4 (bead-, but not antibody-free cells). See "Related Products" for recommendation of products for activation/expansion of T cells.

Required Materials

- Magnet (DynaMag™ potfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes, e.g. HulaMixer® Sample Mixer.
- Cell culture media, e.g. RPMI 1640 w/1% Fetal Bovine Serum (FBS).
- Dynabeads® Mouse CD4 (L3T4).

General Guidelines

- Never resuspend the positively isolated cells in less than 100 µL cell culture media, even if the starting sample is less than 1×10^7 MNC or 1 mL blood.
- The volume of DETACHaBEAD® used relates to the volume of Dynabeads® used for cell isolation; never use less than 10 µL DETACHaBEAD® per 25 µL Dynabeads® used for positive isolation. The amount of DETACHaBEAD® used can be directly scaled up.
- Incubation at 2°C to 8°C will significantly reduce the number of released cells. Incubating at 37°C will not increase release efficiency.
- It is important to remove the DETACHaBEAD® after release, to avoid background staining if using a flow cytometer

Protocol

Prepare Sample

- Perform positive cell isolation with Dynabeads® Mouse CD4 (L3T4) according to that protocol.
- Resuspend the bead-bound cells in 100 µL cell culture media per 25 µL (1×10^7) Dynabeads® Mouse CD4 (L3T4).

Release Mouse CD4⁺ T Cells

The amount of DETACHaBEAD® needed to obtain optimal release varies with the number of Dynabeads® used. This protocol is based on an initial positive cell isolation step from a starting sample of 1×10^7 cells using 25 µL Dynabeads®. Never use less than 10 µL DETACHaBEAD® even when working with less than 25 µL Dynabeads®. When working with >25 µL Dynabeads®, scale up the volumes accordingly (Table 1).

1. Add 10 µL DETACHaBEAD® to the cell sample
2. Incubate for 45 min at room temperature with gentle mixing.
3. Place the tube in a magnet for 1 min.
4. Transfer the supernatant containing released cells to a fresh tube.
5. To obtain residual cells, wash the beads 2–3 times in 500 µL cell culture media and collect the supernatant in a new larger tube.
6. Wash released cells thoroughly by resuspending the cells in a total volume of 10 mL cell culture media and centrifuge for 6 min at 400 × g to remove DETACHaBEAD®.
7. Resuspend the cells in cell culture media or other media, and use in downstream application.

Table 1: Volumes for release of mouse CD4⁺ T cells.

Step	Step description	Volumes per 25 µL Dynabeads®	Volumes per 250 µL Dynabeads®
	Recommended tube size	2 mL	5 mL
	Recommended magnet	DynaMag™-2	DynaMag™-5
1	Cell volume	100 µL	1 mL
1	DETACHaBEAD® volume	10 µL	100 µL
5	Wash cells (cell culture media)	3 × 500 µL	3 × ~4 mL
6*	Remove DETACHaBEAD®	~10 mL	~15 mL

* Transfer the cells to a larger tube and fill the tube up with cell culture media to avoid background staining in the flow analysis.

Description of Materials

DETACHaBEAD® Mouse CD4 is a polyclonal antibody that out-competes the binding of mouse CD4⁺ cells to the Dynabeads® Mouse CD4 (L3T4) (not supplied).

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
DynaMag™-15	12301D
HulaMixer® Sample Mixer	15920D
Dynabeads® Mouse CD4 (L3T4)	11445D
Dynabeads® Mouse T-Activator CD3/CD28	11456D
Dynabeads® FlowComp™ Mouse CD4	11461D

REF on labels is the symbol for catalog number.

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