

Dynabeads[®] Mouse T-Activator CD3/CD28

For activation of mouse T cells

Catalog nos. 11452D, 11453D, 11456D

Store at 2 °C to 8 °C

Publication No. MAN0010201

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Product Contents

Cat. no.	Volume
11456D	1 × 0.4 mL
11452D	1 × 2 mL
11453D	5 × 2 mL

Each product contains 4×10^7 beads/mL in phosphate buffered saline (PBS), pH 7.4, with 0.1% human serum albumin (HSA).

Product Description

This product is intended for activation of mouse T cells, e.g. CD4⁺ T cells or CD8⁺ T cells, T regulatory cells (Treg) (fig. 1).

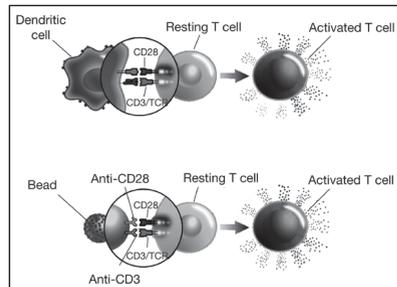


Figure 1: The product mimics *in vivo* T cell activation from antigen-presenting cells (above) by utilizing the two activation signals CD3 and CD28, bound to a three-dimensional bead similar in size to the antigen-presenting cells (below).

Downstream Applications

The activated T cells can be analyzed shortly after activation (for transfection/transduction or to study T cell receptor signaling, proteomics, or gene expression). T cells can be left in culture to differentiate into T helper cell subsets, T cell proliferation or expansion of polyclonal T cells.

For expansion of antigen-specific T cells, see “Related Products”.

Required Materials

- Buffer: PBS with 0.1% bovine serum albumin and 2 mM EDTA, pH 7.4 (PBS with 0.1% BSA).
- DynaMag[™] Magnet (Go to www.lifetechnologies.com/magnets for recommendations).
- Culture medium: Advanced RPMI Medium 1640 with 2 mM L-Glutamine, 10% FCS/FBS and 100 U/mL penicillin/streptomycin, or an equivalent culture medium.
- Heat inactivated Fetal Calf Serum (FCS).
- Recombinant mouse IL-2 (human IL-2 can also be used).
- Flat bottom tissue culture plates or tissue culture flasks.
- Humidified CO₂ incubator.

General Guidelines

- Do not use less than the recommended volume of Dynabeads[®] magnetic beads.
- Always use the recommended pipetting volumes.
- Avoid air bubbles during pipetting.

Protocol

This product allows for easy activation of mouse T cells, without the need for preparing antigen-presenting cells (APCs) or antigen.

Prepare Cells

- See www.lifetechnologies.com/cellisolation for recommended Dynabeads[®] products for positive or negative isolation of all mouse T cells, or specific T cell subsets. Follow the procedure described in the respective package insert.
- Note that for isolation of Treg cells (flow sorting or magnetic bead isolation), it is critical to use an anti-CD25 antibody that does not block the binding of IL-2 if cells are used for expansion. Dynabeads[®] FlowComp[™] Mouse CD4⁺CD25⁺ Treg Cells can be used.
- Prepare cell culture medium.

Wash Dynabeads[®] Magnetic Beads

Wash Dynabeads[®] magnetic beads before use.

1. Resuspend the magnetic beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of magnetic beads to a tube.
3. Add an equal volume of Buffer, or at least 1 mL, and mix (vortex for 5 sec, or keep on a roller for at least 5 min).
4. Place the tube on a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed magnetic beads in the same volume of culture medium as the initial volume of magnetic beads taken from the vial (step 2).

Activate Mouse T cells

1. Start with 8×10^4 purified T cells in 100–200 μ L medium in a 96-well tissue culture plate.
2. Add 2 μ L pre-washed and resuspended Dynabeads[®] magnetic beads to obtain a bead-to-cell ratio of 1:1 (see Table 1).
3. Incubate in a humidified CO₂ incubator at 37°C, according to your specific experimental requirements.
4. Harvest the activated T cells and use directly for further analysis.
5. For flow cytometry applications, remove the beads prior to staining. Place the tube on a magnet for 1–2 min to separate the beads from the solution. Transfer the supernatant containing the cells to a new tube.

Note: To increase recovery of T cells in the supernatant, resuspend the bead/cell suspension thoroughly by pipetting prior to magnetic separation (some cells bind strongly to the beads upon activation and for 2–3 days thereafter). To further increase cell recovery, culture the bead-bound cell fraction overnight and repeat step 5.

Expand Mouse T cells

1. Start with $1\text{--}1.5 \times 10^6$ purified T cells/mL in culture medium in a suitable tissue culture plate or tissue culture flask.
2. Add Dynabeads[®] magnetic beads at a bead-to-cell ratio of 1:1 (see Table 1).
3. Add 30 U/mL rIL-2.
4. Incubate in a humidified CO₂ incubator at 37°C, according to your specific experimental requirements.
5. Examine cultures daily, noting cell size and shape. Cell shrinking and reduced proliferation rate is typically observed in exhausted cell cultures.
6. Count the cells at least twice weekly after thorough resuspension.
7. When the cell density exceeds 2.5×10^6 cells/mL or when the medium turns yellow, split cultures back to a density of $0.5\text{--}1 \times 10^6$ cells/mL in culture medium containing 30 U/mL rIL-2.

Restimulation

Cell cultures showing signs of exhaustion (typically at day 7–10 of expansion) can be restimulated several times by adding fresh Dynabeads® magnetic beads and rIL-2. The CD8⁺ T cells remain cytotoxic after repeated restimulations. Restimulation is typically necessary when cell shrinking and a reduced rate of proliferation is observed. Guidelines for restimulation are provided in Table 2. Optimize for your particular application. Do not use an excess volume of Dynabeads® magnetic beads, as this may inhibit expansion

1. Prior to restimulation, remove the used Dynabeads® magnetic beads by transferring the cells to a suitable tube.
2. Place the tube in the magnet for 1–2 min.
3. Transfer the supernatant containing the cells to a new tube.
4. Split the cultures back to a density of 0.5–1 × 10⁶ cells/mL in culture medium containing 30 U/mL rIL-2 and repeat the “Expand Mouse T Cells” procedure.

Expand Mouse Regulatory T Cells

1. Start with 1–1.5 × 10⁶ cells/mL culture medium in a suitable tissue culture plate (see Table 1).
2. Add Dynabeads® magnetic beads at a bead-to-cell ratio of 2:1 (twice the amount recommended in Table 1).
3. Add rIL-2 at a concentration of 2000 U/mL.
4. Incubate in a humidified CO₂ incubator at 37°C for the length of your specific experiment.
5. Examine cultures daily, noting cell size and shape. Cell shrinking and reduced proliferation rate is typically observed in exhausted cell cultures.
6. Count the cells at least twice weekly after thorough resuspension.
7. Restimulate cells according to the “Restimulation” procedure when the cell density exceeds 2.5 × 10⁶ cells/mL or when the medium turns yellow.

Treg cells retain FoxP3 expression after 2 weeks expansion.

Table 1: Volume recommendations for bead-to-cell ratio = 1:1

Specifications	8 × 10 ⁴ T cells	1 × 10 ⁶ T cells	2 × 10 ⁷ T cells
Type of culture plate/flask	Per well in 96-well plate	Per well in 24-well plate	175 cm ² tissue culture flask
Dynabeads® Mouse T-Activator CD3/CD28	2 µL	25 µL	500 µL
rIL-2	30 U/mL	30 U/mL	30 U/mL
Seeding volume (medium)	100–200 µL	1 mL	20 mL

Table 2: Restimulation guidelines for anti-CD3/CD28-expanded cultures

Cell type	First restimulation*	Subsequent restimulations*
CD4 ⁺ (polyclonal)	8–10 days	8–11 day intervals
CD8 ⁺ (polyclonal)	7–9 days	7–10 day intervals
T cells	7–9 days	10–12 day intervals

* Establish optimal times for your particular cells. Note that these are only generic guidelines.

Description of Materials

Dynabeads® Mouse T-Activator CD3/CD28 are uniform 4.5 µm, superparamagnetic polymer beads coated with an optimized mixture of monoclonal antibodies against the CD3 and CD28 cell surface molecules of mouse T cells. The CD3 antibody is specific for the epsilon chain of mouse CD3, which is considered to be a subunit of the TCR complex. The CD28 antibody is specific for the mouse CD28 co-stimulatory molecule, which is the receptor for CD80 (B7-1) and CD86 (B7-2). Both antibodies are hamster anti-mouse IgGs coupled to the same bead, mimicking *in vivo* stimulation by APCs. Both the bead size and the covalent antibody coupling technology are critical

parameters to allow the simultaneous presentation of optimal stimulatory signals to the T cells in culture, thus allowing their full activation and expansion.

Related Products

A comprehensive range of Dynabeads® products for isolation of T cells and T cell subsets is available. Visit www.lifetechnologies.com/cellisolation.

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
DynaMag™-15	12301D
Phosphate Buffered Saline	10010-023
Advanced RPMI Medium 1640	12633-012
Recombinant human IL-2	PHC0021
Recombinant mouse IL-2	PMC0021

Explanation of Symbols

[REF]	Catalog number
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Limited Use Label License No. 397: ex vivo activation or expansion of T-cells

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