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Dynabeads® Mouse T-Activator CD3/CD28

For activation of mouse T cells

Catalog nos. 11452D, 11453D, 11456D **Store at 2 to 8°C**

Rev. Date: September 2011 (Rev. 004)

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).

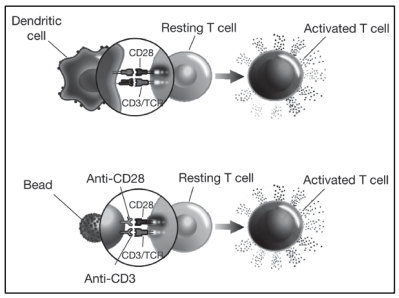

Figure 1: The product mimics *in vivo* T cell activation from antigen-presenting cells (above) by utilizing the two activation signals CD3 and CD28, bound to a three-dimensional bead similar in size to the antigen-presenting cells (below).

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).
- Magnet (DynaMag™): See www.lifetechnologies.com/magnets for magnet recommendations.
- Culture medium: Advanced RPMI Medium 1640 with 2 mM L-Glutamine, 10% FCS/FBS and 100 U/mL penicillin/streptomycin can be used, 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 CO_2 incubator.

General Guidelines

- Resuspend the Dynabeads® according to the "Wash Dynabeads®" section.
- This product should not be used with MPC™-1.
- Never use less than the recommended volume of Dynabeads®.
- Carefully follow the recommended pipetting volumes.
- Avoid air bubbles during pipetting.
- Remove the Dynabeads® and bead-bound cells prior to flow cytometric analysis. Upon activation and for 2–3 days thereafter, some cells bind strongly to the beads. Resuspend the bead/cell suspension thoroughly by pipetting to increase cell recovery, separate on a magnet (after transfer to a suitable tube) and collect supernatant containing the T cells. The bead-bound cell fraction can be cultured overnight and the above process repeated to further increase T cell recovery. When using cells for proteomics or gene expression studies, lyse the cells prior to bead removal.

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®

Wash Dynabeads® before use.

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 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 Dynabeads® in the same volume of culture medium as the initial volume of Dynabeads® taken from the vial (step 2).

Activate Mouse T cells

1. Start with 8×10^4 purified T cells in 100–200 µL medium in a 96-well tissue culture plate.
2. Add 2 µL pre-washed and resuspended Dynabeads® to obtain a bead-to-cell ratio of 1:1 (see Table 1).
3. Incubate in a humidified CO_2 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.

Expand Mouse T cells

1. Start with $1–1.5 \times 10^6$ purified T cells/mL in culture medium in a suitable tissue culture plate or tissue culture flask.
2. Add Dynabeads® at a bead-to-cell ratio of 1:1 (see Table 1).
3. Add 30 U/mL rIL-2.
4. Incubate in a humidified CO_2 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 $0.5–1 \times 10^6$ 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® 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®, as this may inhibit expansion

1. Prior to restimulation, remove the used Dynabeads® 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 × 10^6 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 × 10^6 cells/mL culture medium in a suitable tissue culture plate (10^5 total cells per well in a 96-well plate, 1–1.5 × 10^6 total cells per well in a 24-well plate).
2. Add Dynabeads® at a bead-to-cell ratio of 2:1.
3. Add 2000 U/mL rIL-2.
4. Incubate in a humidified CO_2 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. When the cell density exceeds 2.5 × 10^6 cells/mL or when the medium turns yellow, the cells should be restimulated according to the "Restimulation" procedure.

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	35 × 10^6 T cells*
Type of culture plate/flask	Per well in 96-well plate	Per well in 24-well plate	Per well in 6-well plate
Dynabeads® Mouse T-Activator CD3/CD28	2 µL	25 µL	875 µL
rIL-2	30 U/mL	30 U/mL	30 U/mL
Seeding volume (medium)	100–200 µL	1–2 mL	35–70 mL

* Average number of T cells obtained from one mouse spleen.

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® for isolation of T cells and T cell subsets is available. Visit www.lifetechnologies.com/cellisolation.

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
Dynabeads® Mouse T-Activator CD3/CD28/CD137	11454D
Phosphate Buffered Saline	10010-023
Advanced RPMI Medium 1640	12633-012
Recombinant human IL-2	PHC0021
Recombinant mouse IL-2	PMC0021

REF on labels is the symbol for catalog number.

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