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Dynabeads® Human T-Activator CD3/CD28

For activation of human T cells

Catalog nos. 11131D, 11132D, 11161D **Store at 2 to 8°C**

Rev. Date: September 2011 (Rev. 006)

Product Contents

Cat. no.	Volume
11161D	1 × 0.4 mL
11131D	1 × 2 mL
11132D	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 human T cells, e.g. $CD4^+$ T cells or $CD8^+$ T cells (fig. 1).

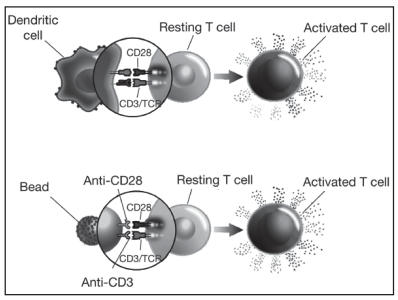

Figure 1: The product mimics *in vivo* T cell activation from antigen-presenting cells (above) by utilizing the two activation signals CD3 and CD28, bound to a three-dimensional bead similar in size to the antigen-presenting cells (below).

Downstream Applications

The activated T cells can be analyzed shortly after activation (for transfection/transduction or to study e.g. 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.

For activation of regulatory T cells or 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. Alternatively, OpTmizer™ T Cell Expansion SFM with 100 U/mL penicillin/streptomycin, or an equivalent culture medium.
- Heat inactivated Fetal Calf Serum (FCS).
- Recombinant human IL-2.
- Flat bottom tissue culture plates or tissue culture flasks of suitable size.
- 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 Dynabeads® and bead-bound cells prior to flow cytometric analysis. Upon activation and for 2–3 days thereafter, some cells will 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 human 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 human T cells, or specific T cell subsets. Follow the procedure described in the respective package insert.
- 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 Human 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 Human T Cells

1. Start with 1–1.5 × 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 × 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 excess Dynabeads® 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.

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

Specifications	8 × 10^4 T cells	1 × 10^6 T cells	50 × 10^6 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® Human T-Activator CD3/CD28	2 µL	25 µL	1,250 µL
rIL-2	30 U/mL	30 U/mL	30 U/mL
Seeding volume (medium)	100–200 µL	1–2 mL	50–100 mL

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

Specifications	8 × 10^4 T cells	1 × 10^6 T cells
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® Human T-Activator CD3/CD28 are uniform 4.5 µm, superpara-magnetic polymer beads coated with an optimized mixture of monoclonal antibodies against the CD3 and CD28 cell surface molecules of human T cells. The CD3 antibody is specific for the epsilon chain of human CD3, which is considered to be a subunit of the TCR complex. The CD28 antibody is specific for the human CD28 co-stimulatory molecule, which is the receptor for CD80 (B7-1) and CD86 (B7-2). Both antibodies are mouse anti-human 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 are available. Visit www.lifetechnologies.com/cellisolation.

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™ – 15	12301D
Dynabeads® Human Treg Expander	11129D
Dynabeads® Human T-Activator CD3/CD28/CD137	11162D
Phosphate Buffered Saline	10010-023
Advanced RPMI Medium 1640	12633-012
OpTmizer™ T Cell Expansion SFM	0080022SA
Recombinant human IL-2	PHC0021

REF on labels is the symbol for catalog number.

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