

# Dynabeads<sup>®</sup> Human T-Activator CD3/CD28/CD137

For activation/expansion of human antigen-specific T cells

Catalog nos. 11162D, 11163D

Store at 2 to 8°C

Rev. Date: September 2011 (Rev. 001)

## Product Contents

Cat. no.	Volume	No. of tests
11162D	0.4 mL	20
11163D	2 mL	100

Each product contains  $5 \times 10^5$  beads/mL in phosphate buffered saline (PBS), pH 7.4, with 0.1% human serum albumin (HSA).

## Product Description

This product is intended for physiological activation of antigen-specific human T cells (e.g. newly isolated antigen-specific T cells, CD4<sup>+</sup> or CD8<sup>+</sup> T cell lines, or clones). Dynabeads<sup>®</sup> Human T-Activator CD3/CD28/CD137 are uniform magnetic beads with size similar to the antigen-presenting cell and represent optimal three-dimensional bead size for efficient T cell activation. Dynabeads<sup>®</sup> are coupled with anti-CD3, anti-CD28, and anti-CD137 antibodies.

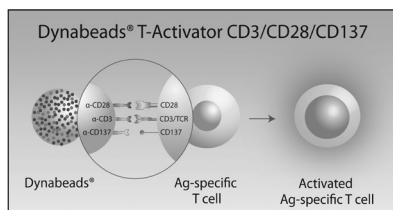


Figure 1: The product mimics *in vivo* T cell activation from antigen-presenting cells by utilizing the three activation signals CD3, CD28, and CD137, bound to a three-dimensional bead similar in size to the antigen-presenting cells.

## Downstream Applications

The T cells activated using T-Activator CD3/CD28/CD137 can be left in culture for expansion of the antigen-specific T cells. Residual beads can easily be removed by a short magnet step and the expanded T cells can be used in any downstream application such as flow cytometry, phenotyping, or other functional assays.

Use Dynabeads<sup>®</sup> Human T-Activator CD3/CD28 for short-term activation experiments or for polyclonal activation of naive T cells.

## Required Materials

- Buffer: PBS with 0.1% bovine serum albumin and 2 mM EDTA, pH 7.4 (PBS with 0.1% BSA). BSA can be replaced by HSA.
- Magnet (DynaMag<sup>™</sup>): See [www.lifetechnologies.com/magnets](http://www.lifetechnologies.com/magnets) for magnet recommendations.
- Culture medium: Advanced RPMI Medium 1640 supplemented with 2% human serum, 2 mM L-Glutamine, and 100 U/ penicillin/streptomycin, or an equivalent culture medium.
- Recombinant human IL-2. Recommend 50 U IL-2/mL, however, optimize each application in the range of 10–100 U IL-2/mL.
- Recombinant human IL-7 is recommended for expansion of CD8<sup>+</sup> T cells.

- Flat or round bottom tissue culture plates or tissue culture flasks.
- Humidified CO<sub>2</sub> incubator.

## General Guidelines

- Resuspend the Dynabeads<sup>®</sup> according to the “Wash Dynabeads” section.
- Carefully follow the recommended pipetting volumes.
- Prior to flow cytometric analysis or use in downstream applications, remove Dynabeads<sup>®</sup> and bead-bound cells by placing the tube in the magnet.
- Optimal bead-to-cell ratios range from 1:5 to 1:10. Optimize the ratio for each application.

## Protocol

This product allows for activation of human antigen-specific T effector/memory cells without the need for preparing antigen-presenting cells (e.g. MNC) or antigen.

## Prepare Cells

- See [www.lifetechnologies.com/cellisolation](http://www.lifetechnologies.com/cellisolation) for recommended Dynabeads<sup>®</sup> products for isolation of T cells. For isolation of antigen-specific T cells, use Dynabeads<sup>®</sup> FlowComp Flexi (Cat. no. 11061D) in combination with anti-CD137 antibodies. Follow the procedure described in the package insert.
- Prepare cells to a concentration of  $1 \times 10^6$  cells/mL.
- Prepare cell culture medium of choice.

## Wash Dynabeads<sup>®</sup>

1. Resuspend the Dynabeads<sup>®</sup> in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Dynabeads<sup>®</sup> to a tube.
3. Add an equal volume of Buffer, at least 1 mL, and mix.
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<sup>®</sup> in the same volume of culture medium as the initial volume of Dynabeads<sup>®</sup> taken from the vial (step 2).

## Activate and Expand T Cells

1. Start with  $1 \times 10^5$  T cells in 100–200  $\mu$ L culture medium in a 96-well tissue culture plate.
2. Add 20  $\mu$ L pre-washed and resuspended Dynabeads<sup>®</sup> to obtain a bead-to-cell ratio of 1:10.
3. Add 50 U/mL rIL-2.  
If expanding CD8<sup>+</sup> T cells, also add 5 ng/mL rIL-7.
4. Incubate in a humidified CO<sub>2</sub> incubator at 37°C changing medium with fresh cytokines every 2–3 days.
5. When the cell density exceeds  $2.5 \times 10^6$  cells/mL or when the medium turns yellow, the cells should be restimulated according to the “Restimulation” procedure.
6. Examine cultures daily, noting cell size and shape.  
**Note:** Cell shrinking and reduced proliferation rate is typically observed in exhausted cell cultures, and occurs typically between days 7–10.

## Restimulation

Restimulate cell cultures showing signs of exhaustion several times by adding fresh Dynabeads® Human T-Activator CD3/CD28/CD137 and cytokines. Re-stimulation is typically necessary when cell shrinking and a reduced rate of proliferation are observed. Guidelines for re-stimulation are provided in Table 1, although we recommend optimization for your particular application. Do not use an excess volume of Dynabeads® Human T-Activator CD3/CD28/CD137 per cell, as this might inhibit expansion.

1. Prior to re-stimulation, remove the used Dynabeads® by transferring the cells to a suitable tube.
2. Place the tube in the magnet for 1–2 minutes until the Dynabeads® have moved to the side of the tube.
3. Transfer the supernatant containing the cells to a new tube.
4. Split the cultures back to a density of  $0.5\text{--}1 \times 10^6$  cells/mL in culture medium containing 50 U/mL rIL-2 (and 5 ng/mL rIL-7 if working with CD8<sup>+</sup> T cells) and repeat the “Expand Mouse T Cells” procedure.

Table 1: Volume recommendations for bead-to-cell ratio = 1:10

Specifications	1 × 10 <sup>5</sup> T cells	1 × 10 <sup>6</sup> T cells	5 × 10 <sup>6</sup> 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® Human T-Activator CD3/CD28/CD137	20 µL	200 µL	1 mL
rIL-2	50 U/mL	50 U/mL	50 U/mL
rIL-7 (for CD8 <sup>+</sup> cells only)	(5 ng/mL)	(5 ng/mL)	(5 ng/mL)
Seeding volume (medium)	100-200 µL	1 mL	4 mL

## Description of Materials

Dynabeads® Human T-Activator CD3/CD28 are uniform 4.5-µm, superparamagnetic polymer beads coated with an optimized mixture of monoclonal antibodies against the CD3, CD28, and CD137 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). The CD137 antibody is specific for the human CD137 co-stimulatory molecule expressed by activated T cells. All antibodies are 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 antigen-specific T cells in culture, thus allowing their full activation and expansion.

## Related Products

Product	Cat. no
DynaMag™-5	12303D
DynaMag – 15	12301D
Dynabeads® FlowComp™ Flexi	11061D
Dynabeads® Human T-Activator CD3/CD28	11131D
Dynabeads® Human Treg Expander	11129D
Phosphate Buffered Saline	10010-023
Advanced RPMI Medium 1640	12633-012
Recombinant human IL-2	PHC0021
Recombinant human IL-7	PHC0075

**REF** on labels is the symbol for catalog number.

Visit [www.lifetechnologies.com/cellisolation](http://www.lifetechnologies.com/cellisolation) or [www.lifetechnologies.com/cellexpansion](http://www.lifetechnologies.com/cellexpansion) for a comprehensive range of Dynabeads® for isolation of T cells and T cell subsets. Products for polyclonal activation/expansion of T cells for mouse and human using Dynabeads® coupled with anti-CD3 and anti-CD28 antibodies are available.

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