

Dynabeads[®] 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 5×10^5 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⁺ or CD8⁺ T cell lines, or clones). Dynabeads[®] 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[®] are coupled with anti-CD3, anti-CD28, and anti-CD137 antibodies.

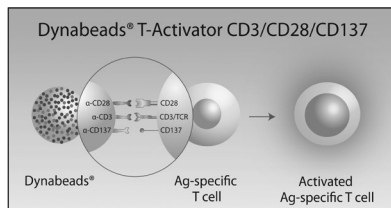


Figure 1: The product mimics *in vivo* T cell activation from antigen-presenting cells by utilizing the three activation signals CD3, CD28, and CD137, bound to a three-dimensional bead similar in size to the antigen-presenting cells.

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[®] 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[™]): See 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⁺ T cells.

- Flat or round bottom tissue culture plates or tissue culture flasks.
- Humidified CO₂ incubator.

General Guidelines

- Resuspend the Dynabeads[®] according to the “Wash Dynabeads” section.
- Carefully follow the recommended pipetting volumes.
- Prior to flow cytometric analysis or use in downstream applications, remove Dynabeads[®] 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 for recommended Dynabeads[®] products for isolation of T cells. For isolation of antigen-specific T cells, use Dynabeads[®] 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 1×10^6 cells/mL.
- Prepare cell culture medium of choice.

Wash Dynabeads[®]

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

Activate and Expand T Cells

1. Start with 1×10^5 T cells in 100-200 μ L culture medium in a 96-well tissue culture plate.
2. Add 20 μ L pre-washed and resuspended Dynabeads[®] to obtain a bead-to-cell ratio of 1:10.
3. Add 50 U/mL rIL-2.
If expanding CD8⁺ T cells, also add 5 ng/mL rIL-7.
4. Incubate in a humidified CO₂ incubator at 37°C changing medium with fresh cytokines every 2-3 days.
5. 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.
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⁺ T cells) and repeat the “Expand Mouse T Cells” procedure.

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

Specifications	1 × 10 ⁵ T cells	1 × 10 ⁶ T cells	5 × 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® 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 ⁺ 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 or 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.

Limited Use Label License

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser’s activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Manufactured by Life Technologies AS. Life Technologies AS complies with the Quality System Standards ISO 9001:2008 and ISO 13485:2003.

SPEC-06907

©2011 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners, except where otherwise stated. Life Technologies AS will not be responsible for violations or patent infringements that may occur with the use of our products.

For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com
www.lifetechnologies.com

 life technologies™