

Dynabeads[®] Mouse T-Activator CD3/CD28/CD137

For activation/expansion of mouse antigen-specific T cells

Catalog nos. 11454D, 11455D

Store at 2 to 8°C

Rev. Date: September 2011 (Rev. 001)

Product Contents

Cat. no.	Volume	No. of tests
11454D	0.4 mL	20
11455D	2 mL	100

Each product contains 5×10^6 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 antigen-specific mouse T cells, e.g. antigen-experienced T cells from tumor-bearing mice, newly isolated antigen-specific T cells, CD4⁺ or CD8⁺ T cell lines, or clones.

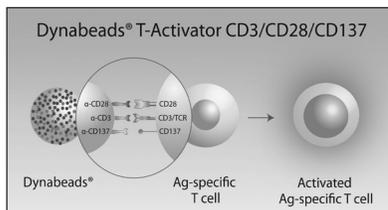


Figure 1: The product mimics *in vivo* T cell activation from antigen-presenting cells by utilizing the three activation signals CD3, CD28, and CD137, bound to a three-dimensional bead similar in size to the antigen-presenting cells.

Dynabeads[®] Mouse 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.

Downstream Applications

The T cells activated using these Dynabeads[®] can be left in culture for expansion of the antigen-specific T cells. Residual beads can easily be removed by a short magnetic step and the expanded T cells can be used in any downstream application such as flow cytometry, phenotyping, adoptive transfer or other functional assays.

For short term activation or polyclonal activation of naive T cells, use Dynabeads[®] Mouse CD3/CD28 (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). BSA can be replaced by HSA.
- Magnet (DynaMag[™]): See www.lifetechnologies.com/magnets for magnet recommendations.
- Culture medium: Advanced RPMI Medium 1640 supplemented with 10 % fetal calf serum, 2 mM L-Glutamine, and 100 U/mL penicillin/streptomycin or an equivalent culture medium.
- Recombinant mouse IL-2. We recommend to use 50 U IL-2 per mL, however optimize each application in the range of 10–100 U IL-2/mL.

- 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, Dynabeads[®] and bead-bound cells should be removed by placing the tube in the magnet.
- Optimal bead-to-cell ratio ranges from 1:1 to 1:10 and optimize the ratio for each application.

Protocol

This product allows for activation of mouse antigen-specific T effector/memory cells without the need for preparing antigen-presenting cells (APCs) 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, we recommend to use Dynabeads[®] FlowComp[™] Flexi with your own antibody.
- Prepare the cells to a concentration of 1×10^6 cells/mL.
- Prepare cell culture medium of choice.

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.
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 1:1.
3. Add 50 U/mL rIL-2.
4. Incubate in a humidified CO₂ incubator at 37°C changing medium with fresh cytokines every 2–3 days.
5. When cell density exceeds 2.5×10^6 cells/mL or when the medium turns yellow, split cultures back to density of $0.5\text{--}1 \times 10^6$ cells/mL in culture medium containing fresh cytokines.
6. Examine cultures daily, noting cell size and shape. Cell shrinking and reduced proliferation rate is observed in exhausted cell cultures, and occurs typically between days 7–10.

Restimulation

Cell cultures showing signs of exhaustion can be restimulated several times by adding fresh Dynabeads® and cytokines (see Table 1). Restimulation is typically necessary when cell shrinking and a reduced rate of proliferation are observed. Optimize for your particular application. Do not use an excess volume of Dynabeads®, as this might inhibit cell 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 $0.5\text{--}1 \times 10^6$ cells/mL in culture medium containing 50 U/mL rIL-2 and repeat the “Activate and Expand T Cells” procedure.

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

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® Mouse T-Activator CD3/CD28/CD137	20 µL	200 µL	1 mL
rIL-2	50 U/mL	50 U/mL	50 U/mL
Seeding volume (medium)	100-200 µL	1 mL	4 mL

Description of Materials

Dynabeads® Mouse T-Activator CD3/CD28/ CD137 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 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). CD137 (4-1BB) is a member of the tumor necrosis factor family and agonistic anti-CD137 antibodies acts as an activating co-stimulatory molecule especially important for effector/memory T cells. Anti-CD137 promotes the survival of T lymphocytes by increasing expression of anti-apoptotic genes bcl-xL and bfl-1 and signaling via CD137 also leads to increased secretion of cytokines (IL-2, IL-4, IFN-γ) which results in augmented proliferation of the 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® Mouse T-Activator CD3/CD28	11452D
Phosphate Buffered Saline	10010-023
Advanced RPMI Medium 1640	12633-012
Recombinant mouse IL-2	PMC0021

REF on labels is the symbol for catalog number.

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

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