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Cat. no. 114.52D
114.53D

Rev. no. 002

Dynabeads® Mouse CD3/CD28 T Cell Expander

For research use only

1. KIT CONTENTS

Dynabeads Mouse CD3/CD28 T Cell Expander Catalog no.	114.52D/114.53D
Dynabeads Mouse CD3/CD28 T Cell Expander*	1x2 ml (114.52D) 5x2 ml (114.53D)

* 4 x 10^7 beads/ml in phosphate buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA).

2. PRODUCT DESCRIPTION

Intended Use

This product is intended for the activation and expansion of mouse T cells, e.g. polyclonal T cells, antigen specific T cells, $CD4^+$ T cells, $CD8^+$ T cells and regulatory T cells (Treg). The expanded cells can be further utilized either for subsequent experimental *in vitro* manipulation, or for adoptive *in vivo* transfer in mouse models of human disease. Applications may include those for the study of infectious disease, transplantation, autoimmunity and cancer.

Principle of T cell Activation and Expansion

Dynabeads Mouse CD3/CD28 T Cell Expander offers a simple method for activation and expansion of T cells that does not require antigen-presenting cells (APCs) or antigen. Simply add Dynabeads Mouse CD3/CD28 T Cell Expander for activation or Dynabeads Mouse CD3/CD28 T Cell Expander plus recombinant IL-2 (rIL-2) for expansion of T cells. Cell cultures showing signs of exhaustion can be re-stimulated several times by adding fresh Dynabeads Mouse CD3/CD28 T Cell Expander and rIL-2. $CD8^+$ T cells remain cytotoxic after repeated re-stimulations. Polyclonal T cells and $CD4^+$ T cells have been successfully expanded for at least 4 weeks; $CD8^+$ T cell cultures typically resist further expansion after 3 weeks. Treg cells retain FoxP3 expression after 2 weeks expansion.

Description of Materials

Dynabeads Mouse CD3/CD28 T Cell Expander 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 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.

Additional Materials Required

- Dynabeads for isolation of desired T cell subset: see www.invitrogen.com/cellisolation for recommended products
- Buffer: Phosphate buffered saline (e.g Gibco cat.no 10010-023) w/0.1% bovine serum albumin, pH 7.4 (PBS w/0.1% BSA)
- Magnet (DynaMag™ or Dynal MPC™): See www.invitrogen.com/magnets-selection for magnet recommendations
- Culture medium: RPMI-1640 (e.g.Gibco cat.no 12633-012) with 2mM L-Glutamin, 10% FCS/FBS and 100 U/ml pencillin/streptomycin or equivalent
- Heat inactivated Foetal Calf Serum (FCS)
- rIL-2
- Flat bottom tissue culture plates or tissue culture flasks of suitable size
- Humidified incubator

▲ Critical notes

- Follow the recommended volumes
- Avoid air bubbles during pipetting
- Do not use the Dynal MPC-1 magnet for any cell isolations

3. PROTOCOL

The protocol describes the activation and expansion of polyclonal T cells, $CD4^+$ or $CD8^+$ T cells isolated from spleen or lymph nodes. In general, it is recommended to use a bead to cell ratio of 1:1, and the cell concentrations given in Table 1. Other bead to cell ratios and cell concentrations can be used following optimization for your particular application. We recommend less intense stimulation for expansion of antigen specific T cells (for example bead to cell ratios 1:3, 1:5 and 1:10 could be tried, choosing an optimal ratio for your application). For Treg expansion, see separate protocol below.

Dynabeads Washing Procedure

Dynabeads should be washed before use.

1. Resuspend the Dynabeads in the vial.
2. Transfer the desired volume of Dynabeads to a tube.
3. Add the same volume of Buffer, or at least 1 ml, and mix.
4. Place the tube in 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 Buffer, as the initial volume of Dynabeads (step 2).

Isolation procedure

See www.invitrogen.com/cellisolation for recommended cell isolation products (positive or negative isolation), and refer to the procedure described in the respective package insert. It is critical for isolation of Treg cells (flow sorting or magnetic bead isolation) to use an anti-CD25 antibody that do not block the binding of IL-2.

Note: Before flow cytometric analysis or use in downstream applications, Dynabeads and bead-bound cells should be removed. During the first days of expansion, some cells will bind strongly to the beads. Resuspend the bead/cell suspension thoroughly by pipetting to increase cell recovery, separate on a magnet (either in a culture plate or following transfer to suitable tubes) 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.

Short term stimulation/activation of mouse T cells

1. Start with 1-1.5 x 10^6 purified T cells/ml culture medium in a suitable tissue culture plate or tissue culture flask.
2. Add Dynabeads Mouse CD3/CD28 T Cell Expander at a bead-to-cell ratio of 1:1.
3. Incubate in a humidified CO_2 incubator at 37°C.
4. Remove the beads at any time before analysis and further use of the cells.

Activation and expansion of polyclonal Mouse T cells

1. Start with 1-1.5 x 10^6 purified T cells/ml culture medium in a suitable tissue culture plate or tissue culture flask.
2. Add Dynabeads Mouse CD3/CD28 T Cell Expander at a bead-to-cell ratio of 1:1.
3. Add 30 U/ml rIL-2
4. Incubate in a humidified CO_2 incubator at 37°C.
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 re-suspension.
7. When the cell density exceeds 2.5 x 10^6 cells/ml or when the medium becomes yellow, split cultures back to a density of 0.5-1 x 10^6 cells/ml in culture medium containing 30 U/ml rIL-2.

Re-stimulation

Re-stimulation is typically necessary when cell shrinking and reduced rate of proliferation are observed. Guidelines for re-stimulation are provided in Table 2, although we recommend optimization for your particular application.

Do not use an excess volume of Dynabeads Mouse CD3/CD28 T Cell Expander, as excess Dynabeads per cell may inhibit expansion.

Before re-stimulation, remove the used Dynabeads by transferring the cells to a suitable tube. Place the tube in a magnet for 1-2 minutes until the Dynabeads are separated. Transfer the supernatant containing the cells to a new tube. Continue as described in step 1 below.

1. Count the cells and resuspend to a density of 1 x 10^6 cells/ml in culture medium with 30 U/ml rIL-2 in a suitable culture plate or tissue culture flask.
2. Add Dynabeads Mouse CD3/CD28 T Cell Expander at a bead-to-cell ratio of 1:1.
3. Add 30 U/ml rIL-2.
4. Incubate in a humidified CO_2 incubator at 37°C.
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 re-suspension.
7. When the cell density exceeds 2.5 x 10^6 cells/ml or when the medium becomes yellow, split cultures back to a density of 0.5-1 x 10^6 cells/ml in culture medium with 30 U/ml rIL-2.

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

	1 x 10^5 T cells	1 x 10^6 T cells	35 x 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 Mouse CD3/CD28 T Cell Expander	2.5 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	35-70ml

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

Table 2: Re-stimulation guidelines for anti-CD3/CD28–expanded cultures

Cell type	First re-stimulation**	Subsequent re-stimulations**
$CD4^+$ (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. Please note that these are only generic guidelines.

Activation and Expansion of Regulatory T cells

1. Start with 1-1.5 x 10^6 cells/ml culture medium in a suitable tissue culture plate (96 well plate: 10^5 total cells/well, 24 well plate: 1-1.5 x 10^6 total cells/well).
2. Add Dynabeads Mouse CD3/CD28 T Cell Expander 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.
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 re-suspension.
7. When the cell density exceeds 2.5 x 10^6 cells/ml or when the medium becomes yellow, split cultures back to a density of 0.5-1 x 10^6 cells/ml in culture medium containing 2000 U/ml rIL-2.

4. GENERAL INFORMATION

Storage and Stability

This kit is stable until the expiry date stated on the label when stored unopened at 2-8°C.

Store opened vials at 2-8°C and avoid bacterial contamination.

Keep Dynabeads in liquid suspension during storage and all handling steps, as drying will result in reduced performance. Resuspend well before use.

Technical Support

Please contact Invitrogen Dynal for further technical information (see contact details).

Warnings and Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated.

Certificate of Analysis/Compliance is available upon request.

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5. REFERENCES

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2. Li J *et al.* (2007) Transfer of in vitro expanded T lymphocytes after activation with dendritomas prolonged survival of mice challenged with EL4 tumor cells. Int J Oncol 31(1): 193-197.
3. Masteller EL *et al.* (2005) Expansion of functional endogenous antigen-specific $CD4^+CD25^+$ regulatory T cells from nonobese diabetic mice. J Immunol 175: 3053-3059.

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