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Dynabeads® Human Treg Expander

Catalog no. 11129D **Store at 2 to 8°C**

Rev. Date: October 2011 (Rev. 004)

Product Contents

Dynabeads® Human Treg Expander at 2 × 10^7 beads/mL concentration are supplied in phosphate buffered-saline (PBS), pH 7.4, with 0.1% human serum albumin (HSA) in a total volume of 2 mL.

Product Description

The Dynabeads® Human Treg Expander is intended for activation and expansion of human Treg cells isolated with the Dynabeads® Regulatory $CD4^+CD25^+$ T Cell Kit. The expanded Treg cells retain their regulatory capacity.

Dynabeads® Human Treg Expander offers a simple expansion method for Treg cells that does not require antigen-presenting cells or antigen. To expand the Treg cells, add Dynabeads® Human Treg Expander and recombinant IL-2 (rIL-2) to the Treg culture.

For polyclonal activation of naive T cells, use Dynabeads® Human T-Activator CD3/CD28. For expansion of antigen-specific T cells, use Dynabeads® Human T-Activator CD3/CD28/CD137 (see "Related Products")

Required Materials:

- Magnet (DynaMag™): See www.lifetechnologies.com/magnets for magnet recommendations.
- Buffer: PBS with 0.1% BSA, pH 7.4.
- Culture medium: 1X OpTmizer™ T-Cell Expansion SFM.
- Round bottom tissue culture plates or tissue culture flasks.
- Humidified CO_2 incubator.
- rIL-2.

General Guidelines

- Use a bead-to-cell ratio of 4:1 and the cell concentrations listed in Table 1. Other bead-to-cell ratios and cell concentrations are suitable following optimization for your application.
- Use at least 95% pure Treg cells for optimal expansion results. Low purity allows $CD8^+$ and $CD4^+CD25^-$ T cells to grow and reduces the number of Treg cells in relative terms.
- If the purity is lower than recommended above, increase the amount of IL-2 up to 1000 U/mL rIL-2 to improve the expansion.

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).

Table 1: Volume recommendations for bead-to-cell ratio = 4:1

Specifications	For 1 × 10^5 Treg cells	For 1 × 10^6 Treg cells
Culture type	Cells/well in 96-well plate	Cells/well in 24-well plate
Dynabeads® Treg Expander	20 µL	200 µL
rIL-2	500 U/mL	500 U/mL
Vol. seeding medium	100 µL	1 mL

Isolate Human Treg Cells

Isolate Human Treg cells using the Dynabeads® Regulatory $CD4^+CD25^+$ T Cell Kit.

Expand Human Treg Cells

Day 0

1. Start with 1 × 10^5 Treg cells in 100 µL culture medium in a round bottom 96-well tissue culture plate.
2. Add 20 µL pre-washed and resuspended Dynabeads® to the cells.
3. Add 500 U/ml rIL-2 to the cells.

Day 1

4. Add 100 µL culture medium containing 500 U/mL rIL-2.

Day 3

5. Resuspend and split the culture in half and add 100 µL culture medium containing 500 U/mL rIL-2 per well.

Days 5–7

6. Split the culture when needed. Examine cultures daily, noting cell size and shape. Cell shrinking and reduced proliferation rate is typically observed in exhausted cell cultures.
7. Count the cells at least twice weekly after thorough resuspension.
8. When the cell density exceeds 2 × 10^6 cells/mL or when the medium becomes yellow, split cultures to a density of 0.5–1 × 10^6 cells/mL in culture medium containing 500 U/mL rIL-2.
9. Grow the cells until the well is half-full (~500,000 cells) and transfer the cells from a 96-well plate to a 24-well plate.

Day 8

10. Remove the Dynabeads® by resuspending the cells and transferring the cells to a suitable tube.
11. Place the tube in a magnet for 1–2 min until the Dynabeads® are separated.
12. Centrifuge the supernatant and resuspend the cell pellet in fresh culture medium containing 100 U/mL rIL-2.
13. Split the cultures when needed and rest the cells until days 21–24 in culture medium with 100 U/mL rIL-2.
14. Incubate the cells in a humidified CO_2 incubator at 37°C.

Restimulation

You can restimulate the cells 15–18 days after the first stimulation or when cell shrinking and reduced rate of proliferation is observed. Restimulation guidelines are listed in Table 2.

Table 2: Volume recommendations for bead-to-cell ratio = 1:1

Specifications	For 1 × 10^5 Treg cells	For 1 × 10^6 Treg cells
Culture type	Cells/well in 96-well plate	Cells/well in 24-well plate
Dynabeads® Treg Expander	5 µL	50 µL
rIL-2	100 U/mL	100 U/mL
Vol. seeding medium	100 µL	1 mL

Before restimulation

1. Count the cells and split the cultures to a density of 1 × 10^6 cells/mL in culture medium.
2. Use the volumes given in Table 2 and follow the protocol below.

Day 0

1. Start with 1 × 10^6 Treg cells in 1 mL culture medium in a 24-well tissue culture plate.
2. Add 50 µL Dynabeads®.
3. Add 100 U/mL rIL-2.

Day 1

4. Add 100 µL culture medium containing 100 U/mL rIL-2 per well.

Day 2 or 3

5. Resuspend and split the culture in half and add 100 µL culture medium containing 100 U/mL rIL-2 per well.

Days 5–7

6. Split the culture when needed. Examine cultures daily, noting cell size and shape. Cell shrinking and reduced proliferation rate is typically observed in exhausted cell cultures.
7. Count the cells at least twice weekly after thorough resuspension.
8. When the cell density exceeds 2 × 10^6 cells/mL or when the medium becomes yellow, split cultures to a density of 0.5–1 × 10^6 cells/mL in culture medium containing 100 U/mL rIL-2.

Day 8

9. Remove the Dynabeads® by resuspending the cells and transferring the cells to a suitable tube
10. Place the tube in a magnet for 1–2 min until the Dynabeads® are separated.
11. Centrifuge the supernatant and resuspend the cell pellet in fresh culture medium containing 100 U/mL rIL-2.
12. Split the cultures when needed and rest the cells until days 21–24 in culture medium with 100 U/mL rIL-2.

Mixed Lymphocyte Reaction (MLR) Assay for Identification of Suppressive Capacity of Expanded Treg Cells

1. Isolate dendritic cells. Culture with TNF-α and LPS for 2 days, and irradiate before use as stimulators in MLR.
2. Establish co-cultures of 5 × 10^4 responder $CD4^+CD25^-$ T cells and 1 × 10^4 irradiated allogenic dendritic cells as stimulators in 96-well U-bottom plates.
3. Add freshly purified or *in vitro* expanded $CD4^+CD25^+$ Treg cells in ratios of 1:1, 1:4, 1:8, 1:16 and 1:32 ($CD25^+$:$CD25^-$ ratio).
4. Pulse the wells on day 6 with ^{3}H-thymidine, and culture for an additional 18 hours before harvest.

Description of Materials

Dynabeads® Human Treg Expander are uniform 4.5 µm, superparamagnetic polystyrene beads coated with an optimized mixture of monoclonal antibodies against the CD3 and CD28 cell surface molecules of human T cells. The CD3 antibody coated on Dynabeads® Human Treg Expander is specific for the epsilon chain of human CD3, 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 coupled to the same bead, mimicking *in vivo* stimulation by antigen presenting cells.

Related Products

Product	Cat. No.
DynaMag™-5	12303D
DynaMag™-15	12301D
Dynabeads® Human T-Activator CD3/CD28/CD137	11162D
Dynabeads® Regulatory $CD4^+CD25^+$ T Cell Kit	11323D
Dynabeads® Human T-Activator CD3/CD28	11131D
OpTmizer™ T-Cell Expansion SFM	0080022SA

REF on labels is the symbol for catalog number.

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