

Dynabeads[®] Human Treg Expander

For research use only

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1 PRODUCT DESCRIPTION

1.1 Intended Use

This product is intended for activation and expansion of human Treg cells isolated with the Dynal CD4⁺CD25⁺ Treg Kit (Cat. no. 113.23D). The expanded Treg cells retain their regulatory capacity (1-3).

1.2 Principle of expansion

Dynabeads Human Treg Expander offers a simple method for expansion of Treg cells that does not require antigen-presenting cells or antigen. Just add Dynabeads Human Treg Expander and recombinant IL-2 (rIL-2) to the Treg culture to expand the Treg cells. Cell cultures showing signs of exhaustion can be re-stimulated by adding fresh beads and rIL-2.

Ready-to-use Dynabeads Human Treg Expander offer the first artificial antigen-presenting cells to provide simultaneous signals to TCR/CD3 and CD28 for full activation and expansion of human Treg cells.

1.3 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 sub-

unit 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.

Materials Supplied

- 2 ml Dynabeads Human Treg Expander
- 2 x 10⁷ beads/ml in phosphate buffered saline (PBS), pH 7.4, w/ 0.1% human serum albumin (HSA).

Additional materials required

Materials that are not included, but are needed to perform the entire protocol:

- Magnet: Any Dynal MPC[™]
- Buffer 1: PBS w/ 0.1% BSA, pH 7.4
- Culture Medium: X-vivo (or equivalent) with 2mM L-glutamine, 5% human AB serum, 100 U/ml penicillin/streptomycin (or equivalent)
- Round bottom tissue culture plates or tissue culture flasks of suitable size
- Humidified CO₂ incubator
- Dynal CD4⁺CD25⁺ Treg Kit (Cat. no. 113.23D)
- rIL-2

2. PROTOCOLS

2.1 Technical Advice

It is generally recommended to use a bead-to-cell ratio of 4:1 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.

At least 95% pure Treg cells will give the most optimal expansion results. Low purity will allow 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, the expansion results might be sub-optimal. To improve the expansion results, the amount of IL-2 can be increased up to 1000 U/ml rIL-2.

2.2 Dynabeads Washing Procedure

Dynabeads should be washed before use with the aid of a magnet.

1. Resuspend the Dynabeads in the vial.
2. Transfer the desired volume of Dynabeads to a tube.
3. Add the same volume of Buffer 1 as the initial volume of Dynabeads, or at least 1 ml, and mix.
4. Place the tube in a magnet for 1-2 minutes until the Dynabeads are separated; discard the supernatant. Remove the tube from the magnet.
5. Resuspend the washed Dynabeads in the same volume of Buffer 1 as the initial volume of Dynabeads.

Table 1

Volume/Number of Treg Cells	1 x 10 ⁵ Treg Cells	1 x 10 ⁶ Treg Cells
Type of Culture	Per well in 96-well tissue culture plate	Per well in 24-well tissue culture plate
Dynabeads Treg Expander	20 μl	200 μl
rIL-2	500 U/ml rIL-2	500 U/ml rIL-2
Seeding Volume (medium)	100 μl	1 ml

2.3 Isolation of Human Treg Cells

For isolation of human Treg cells it is recommended to use the Dynal CD4⁺CD25⁺ Treg Kit (Cat no. 113.23D).

2.4 Expansion of Human Treg Cells

Day 0:

Start with 1 x 10⁵ Treg cells in 100 μl Culture Medium in a round bottom 96 well tissue culture plate.

Add 20 μl Dynabeads Human Treg Expander.

Add 500 U/ml rIL-2.

Day 1:

Add 100 μl Culture Medium containing 500 U/ml rIL-2.

Day 3:

Resuspend and split the culture in half and add 100 μl Culture Medium containing 500 U/ml rIL-2 per well.

Day 5-7:

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. Count the cells at least twice weekly after thorough resuspension.

When the cell density exceeds 2 x 10⁶ cells/ml or when the medium becomes yellow, split cultures to a density of 0.5 - 1.0 x 10⁶ cells/ml in Culture Medium containing 500 U/ml rIL-2.

Grow the cells until the well is half-full (~500,000 cells) and transfer the cells from a 96 well to a 24 well plate.

Day 8:

Remove the Dynabeads by resuspending the cells and transferring the cells

to a suitable tube. Place the tube in a magnet for 1-2 minutes until the Dynabeads are separated. Centrifuge the supernatant and resuspend the cell pellet in fresh Culture Medium containing 100 U/ml rIL-2.

Split the cultures when needed and rest the cells until Day 21-24 in culture medium with 100 U/ml rIL-2.

Incubate in a humidified CO₂ incubator at 37°C.

2.5 Re-stimulation of Human Treg Cells

The cells can be re-stimulated 15-18 days after the first stimulation, or when cell shrinking and reduced rate of proliferation is observed. Guidelines for re-stimulation are provided in Table 2.

Before re-stimulation:

1. Count the cells and split the cultures to a density of 1 x 10⁶ cells/ml in Culture Medium.
2. Use the volumes given in Table 2 and follow the protocol below.

Day 0:

Start with 1 x 10⁶ Treg cells in 1 ml Culture Medium in a 24 well tissue culture plate.

Add 50 μl Dynabeads Treg Expander.

Add 100 U/ml rIL-2.

Day 1:

Add 100 μl Culture Medium containing 100 U/ml rIL-2 per well.

Day 2 or 3:

Resuspend and split the culture in half and add 100 μl Culture Medium containing 100 U/ml rIL-2 per well.

Day 5-7:

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. Count the cells at least twice weekly after thorough resuspension.

When the cell density exceeds 2 x 10⁶ cells/ml or when the medium becomes yellow, split cultures to a density of 0.5 - 1.0 x 10⁶ cells/ml in Culture Medium containing 100 U/ml rIL-2.

Day 8:

Remove the Dynabeads by resuspending the cells and transferring the cells to a suitable tube. Place the tube in a magnet for 1-2 minutes until the Dynabeads are separated. Centrifuge the supernatant and resuspend the cell pellet in fresh Culture Medium containing 100 U/ml rIL-2.

Split the cultures when needed and rest the cells until Day 21-24 in culture medium with 100 U/ml rIL-2.

Table 2

Volume/Number of Treg Cells	1 x 10 ⁵ Treg Cells	1 x 10 ⁶ Treg Cells
Type of Culture	Per well in 96-well tissue culture plate	Per well in 24-well tissue culture plate
Dynabeads Treg Expander	5 µl	50 µl
rIL-2	100 U/ml rIL-2	100 U/ml rIL-2
Seeding Volume (medium)	100 µl	1 ml

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

Isolate dendritic cells and culture with TNF- α and LPS for 2 days and irradiate before use as stimulators in MLR.

Establish co-cultures of 5 x 10⁴ responder CD4⁺CD25⁻ T cells and 1 x 10⁴ irradiated allogenic dendritic cells as stimulators in 96-well U-bottom plates. 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). Pulse the wells on day 6 with ³H-thymidine, and culture for an additional 18 hours before harvest.

3. GENERAL INFORMATION

Invitrogen Dynal AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

Storage/Stability

This product 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 AS for further technical support (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.

Follow appropriate laboratory guidelines.

This product contains 0.02% sodium azide as a preservative, which is cytotoxic. **Avoid pipetting by mouth!** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide build up.

Certificate of Analysis (CoA) is available upon request.

Material Safety Data Sheet (MSDS) is available at <http://www.invitrogen.com>.

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Invitrogen Corporation,
1600 Faraday Avenue, Carlsbad,
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4. REFERENCES

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- Hoffmann P *et al.* (2004) Large-scale *in vitro* expansion of polyclonal human CD4⁺CD25^{high} regulatory T cells. *Blood* 104(3): 895-903.

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