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CTS™ Dynabeads™ Treg Xpander

Catalog No. 46000D **Store at 2°C to 8°C**

Publication No. MAN0018604 **Rev.** B.0

Product contents

Cat. No.	Volume
46000D	10 mL

CTS™ Dynabeads™ Treg Xpander contains 2×10^8 beads/mL in phosphate buffered-saline (PBS), pH 7.4, with 0.1% recombinant human serum albumin (recombinant HSA), sufficient for activating and expanding 5×10^8 regulatory T cells (Treg cells).

Product description

CTS™ Dynabeads™ Treg Xpander is intended for *ex vivo* activation and expansion of human Treg cells for cell-based therapy.

CTS™ Dynabeads™ Treg Xpander is a magnetic bead conjugated with anti-CD3 and anti-CD28 antibodies at a specific ratio.

Treg cells activated by CTS™ Dynabeads™ Treg Xpander can be expanded 100–1000 fold over a 9–14 day culture period with the option of a re-stimulation step during the process.

Required materials

For clinical research procedures, the principal investigator is responsible for ensuring that use of all procedures, reagents, and equipment follow applicable guidelines, standards, and regulations. The materials and equipment in the following list are recommended for use with CTS™ Dynabeads™ Treg Xpander procedures. Alternative materials and equipment may be used.

- DynaMag™-5, DynaMag™-15, or DynaMag™-50 Magnet (see **thermofisher.com/magnets**)
- CTS™ OpTmizer™ T-Cell Expansion SFM
- CTS™ Recombinant Human IL-2
- L-Glutamine (200 mM)
- CTS™ Immune Cell SR

General guidelines

- Follow universal precautions when working with human serum, plasma, or blood products.
- The purity of the starting material affects the function of expanded Treg cells. Higher purity results in a higher percentage of FoxP3 expression and suppressive function in the final Treg cell product.
- Carefully follow the recommended pipetting volumes and incubation times. Using lower volumes of Treg Xpander than recommended may yield lower fold expansion and FoxP3 expression.
- Because sample source and method of cell or blood collection may vary, specific modifications of the respective procedure to maximize cell recovery and viability may be required.
- Any application of *ex vivo* processed target cells is exclusively within the responsibility of the user.

Prepare media

Equilibrate the medium to room temperature before use.

Expansion Medium

Add CTS™ OpTmizer™ Expansion Supplement and L-Glutamine to CTS™ OpTmizer™ T-Cell Expansion Basal Medium according to user guide.

Complete Expansion Medium

Add 300 IU/mL of CTS™ IL-2 Recombinant Human Protein (rIL-2) and 5% of CTS™ Immune Cell SR to the Expansion Medium.

Wash Dynabeads™ magnetic beads

Wash CTS™ Dynabeads™ Treg Xpander beads before use.

1. Resuspend the beads to a homogenous mixture in the vial (i.e., vortex for >30 sec, or tilt and rotate for 5 min).
   Note: Do not allow beads to sediment before next step.
2. Immediately transfer the desired volume of beads to a tube.
3. Add an equal volume of Expansion Medium, 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 beads in the same volume of Expansion Medium as the initial volume of beads taken from the vial (step 2). Avoid formation of air bubbles during pipetting.

Isolate human Treg cells

Isolate fresh human Tregs prepared from Ficoll separated whole blood, elutriated apheresis, or samples derived from sources such as cord blood or thymus.

Expand human Treg cells

Use a 4:1 bead-to-cell ratio for CTS™ Dynabeads™ Treg Xpander beads and Treg cells for the initial stimulation and a 1:1 ratio for re-stimulation at day 9.

The following protocol is performed with 2×10^5 Treg cells/well.

Day 0

1. Prepare Treg cells at a concentration of 2×10^6 cells/mL in Expansion Medium. 100 µL of cells is required for each well of a 48-well plate.
2. Dilute washed and resuspended CTS™ Dynabeads™ Treg Xpander beads to a concentration of 1×10^7 beads/mL in Expansion Medium.
3. Add beads and cells at a 4:1 ratio to wells of a 48-well flat bottom cell culture plate using the volumes listed in the following table.

Component	Volume
Expansion Medium	170 µL
2×10^6 cells/mL	100 µL
CTS™ Dynabeads™ Treg Xpander beads (1×10^7 beads/mL)	80 µL
Total volume	350 µL

4. Incubate the cells in a humidified CO_2 incubator at 37°C.

Day 2

5. Add 200 µL Complete Expansion Medium.
6. Incubate the cells in a humidified CO_2 incubator at 37°C.
   IMPORTANT! Cells and beads are forming rosettes and should not be disturbed during this incubation period.

Day 4–9

7. Examine cultures daily using a microscope and record observations on density and cell clusters.
8. If cell density is <2–3 × 10^8 cells/mL, remove half of the medium from each well, without disturbing the cells. Replace the removed volume with fresh Complete Expansion Medium and resuspend the cells.
   If cell density reaches >2–3 × 10^8 cells/mL, resuspend the cells and transfer to a larger well format with addition of fresh Complete Expansion Medium.

Product Use: For research use or non-commercial manufacturing of cell-based products for clinical research.

Caution: Not intended for direct administration into humans or animals.

Day 9

9. Harvest the cells into tubes for magnetic separation.
10. Place the tube in an appropriate DynaMag™ Magnet for 1–2 min until the beads are separated.
11. Transfer the supernatant (containing the cells) to a new tube.
12. Count the cells and dilute the cultures to a density of 1×10^6 cells/mL in Complete Expansion Medium.
13. Add washed and resuspended Treg Xpander beads to the cells at a ratio of 1:1, then seed the cells in an appropriately sized plate or flask.
14. Incubate the cells in a humidified CO_2 incubator at 37°C.

Days 11–12

15. Examine cultures daily using a microscope and record observations on density and cell clusters.
16. If needed, add fresh Complete Expansion Medium in each well (see step 8).

Day 14

17. Harvest the cells and remove the beads (see steps 9–11).

USA (Master File)

CTS™ Dynabeads™ Treg Xpander is available for use in clinical trials under an approved IND.

A Master File is held with the United States Food & Drug Administration (FDA), which will assist users with their application for FDA approvals on their clinical trials. If cross referencing the Master File is of interest to an Investigational New Drug (IND) Application or other applications, please contact Life Technologies AS with the sponsor's and/or investigator's full name and address, along with project name and aim. This information is required by Life Technologies AS to issue a Letter of Authorization, informing the FDA who has been authorized to cross-reference the Master File for their IND application.

Description of Materials

CTS™ Dynabeads™ Treg Xpander are uniform superparamagnetic, nonpyrogenic polystyrene beads with affinity purified mouse anti-human CD3 and CD28 monoclonal antibodies covalently bound to the surface.

Related Products

Product	Cat. No.
DynaMag™-5 Magnet	12303D
DynaMag™-15 Magnet	12301D
DynaMag™-50 Magnet	12302D
CTS™ DynaMag™ Magnet [1]	12102
HulaMixer™ Sample Mixer	15920D
CTS™ OpTmizer™ T-Cell Expansion SFM	A1048501, A1048503
CTS™ OpTmizer™ T-Cell Expansion SFM, no phenol red	A3705001, A3705003
CTS™ Recombinant Human IL-2	CTP0021
L-Glutamine (200 mM)	25030
CTS™ GlutaMax™ Supplement	A1286001
CTS™ Immune Cell SR	A2596102

[1] For bag-based expansion systems.

Limited Use Label License No. 647

Ex vivo activation or expansion of human T-cells

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