

Dynabeads™ CD3/CD28 CTS™

Catalog no. 40203D

Store at 2°C to 8°C

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Rev. D.0

Product contents

Cat. No.	Volume
Dynabeads™ CD3/CD28 CTS™	10 mL

CTS™ Dynabeads™ CD3/CD28 contains 4×10^8 beads/mL in phosphate buffered saline (PBS), pH 7.4, with 0.1% recombinant human serum albumin (recombinant HSA).

Product description

Dynabeads™ CD3/CD28 CTS™ are intended for separation (1, 2), activation (3), and *ex vivo* expansion (1, 4, 6) of human T cells for cell-based clinical research.

Dynabeads™ CD3/CD28 CTS™ are produced according to cGMP (21 CFR Part 820) in a validated Class 100 clean room using gamma irradiated Dynabeads™ magnetic beads. All manufacturing processes are validated. Sterility and endotoxin tests are performed according to current United States Pharmacopeia (USP). Dynabeads™ CD3/CD28 CTS™ offer a simple method for isolation (1, 2), activation (3), and expansion (1, 6) of human T cells. CD3⁺ T cells can be separated and concentrated from an apheresis product by magnetic cell separation using Dynabeads™ CD3/CD28 CTS™ (1, 2). Following separation, the CD3⁺ T cells are cultured in the presence of the beads. By combining anti-CD3 and anti-CD28 antibodies on Dynabeads™ magnetic beads, the beads will provide both the primary and co-stimulatory signals required for activation and expansion of T cells (5). The activated T cells have been shown to produce IL2, GM-CSF, IFN- γ , and TNF- α (1, 5, 6). T cells activated with these Dynabeads™ magnetic beads can be expanded 100–1000 fold over a 9–14 day culture period. The T cell expansion process can be scaled up using a bioreactor-based process (6). It has been shown that the T cell expansion protocol can be optimized to include expansion of antigen-specific T cells (7–12).

Required materials not supplied

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 the Dynabeads™ CD3/CD28 CTS™ procedures. Alternative materials and equipment may be used. Materials that are not included, but are required to perform the procedures:

- DynaMag™ Magnet (The DynaMag™ CTS™ magnet is recommended for larger volumes where the PBMC and Dynabeads™ CD3/CD28 CTS™ are in closed bags. See [thermofisher.com/magnets](https://www.thermofisher.com/magnets) for additional recommendations)
- CTS™ DPBS (must be calcium- and magnesium-free)
- Recombinant human serum albumin (HSA)
- CTS™ OpTmizer™ T-Cell Expansion SFM (serum free medium for support the culture and expansion of human T cells) or CTS™ AIM V™ Medium Therapeutic Grade (serum free cell expansion medium)
- CTS™ IL-2 Recombinant Human Protein
- 1-L Bags (Terumo™ or CellGenix™ VueLife™) for magnetic separation
- 3-L Culture Bags (CellGenix™ VueLife™) for cell expansion
- Sampling site coupler with female luer (Charter Medical)
- Terumo™ TSCD™ Sterile Tubing Welder
- 40–80µm In-line Transfusion Filter (Pall)
- 10-lead harness sets (compatible with Terumo™ SCD 312 welder), (Charter Medical)
- Hemostats tube clamp

- COBE™ 2991 Cell Washer Disposable Set (COBE/Gambro) or Cytomate™ Disposable Set (Baxter)
- Sample mixer allowing tilting and rotation at 1–3 rpm (e.g., HulaMixer™ Sample Mixer)
- Plasma thawing device (e.g., ThermoGenesis™ MT202)
- Bioreactor (e.g., WAVE Bioreactor™)

Prepare buffers and growth media

Buffer 1:

DPBS CTS™ without Ca^{2+} and Mg^{2+} , with 1% HSA.

Incomplete Medium:

OpTmizer™ CTS™ T-Cell Expansion SFM serum-free 1X formulation
designed to support the culture and expansion of human T cells
(or equivalent)

or

AIM V™ Medium CTS™ Therapeutic Grade serum free cell expansion medium.

Complete Medium:

Prepare fresh Complete Medium every week by adding 200 IU/mL IL-2 to the Incomplete Medium. Equilibrate Complete Medium to room temperature prior to use. Improved expansion might be obtained by adding 2–5% human AB serum.

Important guidelines

- Follow universal precautions when working with human serum, plasma, or blood products.
- All human samples must be treated as a potential source of HIV, HBV, and other bloodborne pathogens.
- Gloves and a laboratory coat must be worn when working with human samples.
- Materials contaminated with blood products must be decontaminated by an approved chemical method and disposed of in labeled biohazard containers.
- Solution transfers not performed in a closed system, such as spike connections and open containers, must be performed under a Class 100 biological safety cabinet (BSC) using aseptic techniques.
- This protocol describes activation (3) and expansion (1, 6) of human T cells from cryopreserved apheresed products.
- Cultures may also be initiated from non-cryopreserved fresh samples, or samples derived from sources other than apheresis, such as Ficoll separated whole blood (1, 3), cord blood (2), or bone marrow (13).
- As each sample source and method of T cell or blood collection may vary, procedures may require specific modifications to maximize cell recovery, viability, activation, and expansion. Such modifications must be determined empirically.

General guidelines

Visit thermofisher.com/samplepreparation for recommended sample preparation procedures.

- Avoid air bubbles (foaming) during pipetting.
- Never use less than the recommended volume of Dynabeads™ magnetic beads.
- Carefully follow the recommended pipetting volumes and incubation times.
- Keep all buffers cold.
- Dynabeads™ CD3/CD28 CTS™ is used in customer-specific applications. The customer-specific applications should be qualified by the customer by the use of ≤6 donors to understand the donor variation and the impact on the effect and the safety.

Protocol

Notes in the following protocol indicate areas where modifications should be considered for specific circumstances.

Wash Dynabeads™ magnetic beads

1. Resuspend the Dynabeads™ magnetic beads in the vial (i.e., vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Dynabeads™ magnetic beads to a tube.
3. Add the same volume of Buffer 1, or at least 1 mL, and resuspend.
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™ magnetic beads in the same volume of Buffer 1 as the initial volume of Dynabeads™ magnetic beads (step 2).

Starting materials

The preferred starting material is cryopreserved human PBMC obtained from leukapheresis product (6). The starting material can also be enriched for specific T cell subsets, such as CD4⁺ T cells (14) or CD25⁺ T cells (15, 16, 17). For optimal T cell activation and expansion it is recommended that freshly collected samples are cryopreserved and thawed prior to use.

Note: Cryopreservation and subsequent thawing facilitates lysis of granulocytes and other cell types that can suppress T cell activation and expansion. This may be particularly important when working with samples from patients in certain disease states where granulocyte counts are elevated.

Alternatively, PBMC from freshly obtained leukapheresis product may be activated and expanded without cryopreservation. For maximum activation and expansion of T cells in PBMC, magnetically capture CD3⁺ T cells prior to culture initiation (1, 2) (see "Magnetic separation and expansion of CD3⁺ T cells"). Magnetic concentration is not required if T cells or T cell subsets have been enriched prior to activation and expansion (e.g., purified CD4⁺ T cells) (14).

Thaw and wash cryopreserved apheresis cells

1. Remove the required number of bags containing cryopreserved apheresis cells from liquid nitrogen vapor storage.
2. Thaw the bag(s) in a plasma thawing device or by equivalent methods.
3. To prevent cell clumping, add anticoagulant solution aseptically to thawed cells to a final concentration of 10%.
4. Slowly dilute the cell suspension 1:1 in Buffer 1.
5. Wash the cells in Buffer 1 according to the cell washer manufacturer's recommendations.
6. Resuspend the cells in 50–60 mL of Buffer 1. If the volume exceeds 60 mL, perform a centrifugation step to reduce the volume.
7. Incubate the cells for 60 min in Buffer 1 at room temperature to allow dead or dying cells to aggregate and subsequently be removed via a blood filter as described below.
8. Filter the cells through an In-line Transfusion Filter with cut-off between 40–80 μm . The cells are now ready for further processing.

Remove 1 mL of the sample from the leukapheresis bag. Calculate the number of CD3⁺ T cells by flow cytometry, and determine the viability of the cells with trypan blue staining.

Magnetic separation and expansion of CD3+ T cells

This procedure is for separation (1, 2) and expansion (1, 6) of 5 × 10⁸ CD3⁺ T cells. Adjust the volumes accordingly when using higher/lower cell numbers. Magnetic pre-selection of CD3⁺ T cells can improve subsequent T cell expansion (1, 2). The recommended standard procedure described below uses a ratio of three Dynabeads™ CD3/CD28 CTS™ per CD3⁺ T cell.

Note: If the starting sample contains less than 25% CD3⁺ T cells, replace Buffer 1 with Incomplete Medium in the following procedures.

1. Dilute the cells to approximately 1 × 10⁷ CD3⁺ T cells/mL in Buffer 1. For the standard procedure of processing 5 × 10⁸ CD3⁺ T cells, dilute the sample in 50–60 mL of Buffer 1.
2. Use a 1-L bag for CD3⁺ T cell separation. Add 100 mL of air to the bag.
3. Add 5 × 10⁸ CD3⁺ T cells to the 1-L bag in 50–60 mL Buffer 1.
4. Add 4.0 mL (corresponding to 1.6 × 10⁹ Dynabeads™ magnetic beads) of washed Dynabeads™ CD3/CD28 CTS™ per 5 × 10⁸ CD3⁺ T cells and immediately proceed to the next step.
5. Place the bag on a cell mixer and mix for 30 min at room temperature to gently mix the cells and the Dynabeads™ magnetic beads (1–3 rpm).
Note: If the starting sample contains less than 25% CD3⁺ T cells it may be beneficial to mix the sample for 1–2 hours in Incomplete Medium instead of Buffer 1. Optimize the mixing temperature between 4°C to 25°C for each application.
6. Prepare 150 mL of Buffer 1 in a 300-mL bag.
7. After mixing, remove the 1-L bag from the mixer and drain 150 mL of Buffer 1 into the bag.
Note: Handle the 1-L bag very gently, to not disrupt the bead/cell complexes. Place the 1-L bag directly on the DynaMag™ CTS™ magnet. Adjust the magnet to a 60° angle.
8. Leave the bag on the DynaMag™ CTS™ magnet for 1 min to capture the bead-bound CD3⁺ T cells. While on the magnet, open the 1-L bag containing the captured cells and drain waste fluid out in waste bag via gravity. Remove the bag containing the captured cells from the magnet immediately after all waste fluid has been drained.
9. Immediately add approximately 300 mL Complete Medium to the 1-L bag containing the captured cells and gently resuspend the cell/bead complexes.
10. Obtain a 3-L bag.
11. Transfer the cell/bead complexes from the 1-L bag into a 3-L bag. Wash the 1-L bag with Complete Medium and transfer the residual cells to the 3-L bag.
12. Repeat media wash of the 1-L bag and transfer the residual cells to the 3-L bag until the volume in the 3-L bag has reached 1000 mL.
13. Place the 3-L bag in an incubator at 37°C/5% CO₂ until Day 3 of culture.
Note: Using a bioreactor (e.g., WAVE Bioreactor™), will increase expansion efficiency via perfusion and improved aeration (rocking). Where as typical cell densities rarely exceed 2–3 × 10⁶ T cells/mL in static cultures, bioreactor systems can readily maintain viable T cells at densities of 2–4 × 10⁷ > T cells/mL.
14. Collect a sample of the non-captured cell fraction and manually count the number of non-captured cells.
15. Stain the non-captured cells for CD3 expression and evaluate by flow cytometry to calculate depletion efficacy.

Count and split cultures

Evaluate the cell concentration daily beginning on day 3 of culture.

1. Gently mix the bag to help dissociate cell/bead complexes and to resuspend the cells before removing 1–2 mL cell suspension for counting.
2. Again, mix the sample well to resuspend cells so as to ensure maximum dissociation of beads from cells. This will improve cell count accuracy.
3. Take an aliquot of cells and mix 1:1 with trypan blue staining solution and manually count on a hemacytometer (do not remove the Dynabeads™ magnetic beads before counting). Determine cell density and viability.
Note: Insufficient mixing of bead/cell complexes may result in cell count underestimates as they will not migrate efficiently under hemocytometer coverslips.
4. Stain cells for CD3 expression and evaluate by flow cytometry to calculate the number of CD3⁺ T cells in the bag.
5. Determine the total cell volume by weighing the bag.
6. When CD3⁺ T cell density exceeds 1 × 10⁶ cells/mL, dilute the cells to approximately 0.5 × 10⁶ CD3⁺ T cells/mL in Complete Medium.
7. Split the cultures to new 3-L bags when needed.
Note: T cell growth typically slows as T cell concentrations increase above 1–2 × 10⁶ T cells/mL, so adjust T cell numbers to ~0.5 × 10⁶/mL to help maintain the cells in log phase growth.
8. Repeat counting of cells daily and dilute cells in fresh Complete Medium to 0.5 × 10⁶/mL.

Harvest expanded CD3+ T cells

1. Harvest cells on an optimal day for your application (usually day 8–12).
2. Remove the 3-L bags from the incubator. Remove a sample from a representative number of bags for cell count and FACS analysis. Perform the cell counts as described above.
3. Remove the beads by passing the cell culture over the DynaMag™ CTS™ magnet using gravity driven flow. Determine the angle of the primary magnet of DynaMag™ CTS™ magnet empirically between 0°–60°. To achieve a flow rate of up to 150 mL/min, the bags containing cells and beads must be suspended from the pole so that the fluid level is 85–90 cm above the cell collection bags.
4. Concentrate the cells and wash using a cell washer.
5. Perform a final bead removal twice by placing the bag on the DynaMag™ CTS™ magnet. Adjust the magnet to a 60° angle. After 1 min, drain the cells into a new bag via gravity.
6. Determination of residual beads can be performed as described in reference 18.

Cryopreserve expanded CD3+ T cells

1. Prepare cryopreservation medium and cryopreserve the expanded T Cells.
2. Store the final product of expanded T cells in the vapor phase of a liquid nitrogen storage unit.

Guidelines for procedures incorporating gene transduction

Typically, for all culture conditions described earlier, T cells from normal donor samples begin cycling and start to divide between day 2 and 3 of culture (5, 9). Day 1, 2, and/or 3 are recommended as optimal days for transduction using lentivirus-based vectors (19). Magnetic removal of beads prior to transduction diminishes overall cell expansion, but should not affect the viability. Leaving beads in during the retroviral transduction process should be acceptable for most transduction applications.

Note: T cells obtained from patients with various diseases and/or undergoing various treatments may be slower at entering cell cycle and cell division may not commence until 1, 2, or even 3 days later than typically observed for samples from healthy donors. For example, T cells from patients with HIV infection may be slower to start cell cycling, as may be samples from patients undergoing chemotherapy, or patients with certain kinds of cancer (e.g., chronic lymphocytic leukemia) (1, 9, 20–22). Thus, it is important to monitor T cell activation markers, such as CD25, as well as cell division to determine optimal splitting schedules and timing for gene modification.

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Ex vivo activation or expansion of human T-cells

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Note: This product was previously branded Xcyte™ Dynabeads™ and Dynabeads™ ClinExVivo™ CD3/CD28.

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General Information

Certification

Life Technologies AS complies with the Quality Systems Standard ISO 9001:2008 and ISO 13485:2012 with the following scope:
"Design, development, manufacturing, and sales of products either used as standalone *in vitro* diagnostic (IVD) products, or for further manufacturing of OEM products for IVD markets.

Design, development, manufacturing, and sales of products intended for *ex vivo* separation of human cells, and for cell-based clinical research"

In the United States, Dynabeads™ CD3/CD28 CTS™ is available for use in clinical trials under an approved IND or IDE.

USA (Master File)

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 Authorisation, informing the FDA who has been authorised to cross-reference the Master File for their IND application.

Description of materials

Dynabeads™ CD3/CD28 CTS™ 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™ CTS™ Magnet	12102
MPC™-1 Magnet	12001D
DPBS CTS™ without calcium chloride, without magnesium chloride	A1285601
HulaMixer™ Sample Mixer	15920D
OpTmizer™ CTS™ T-Cell Expansion	A10485-01
IL-2 CTS™ Recombinant Human	CTP0021 or CTP0023
AIM V™ Medium CTS™ Medium	0870112DK

[REF] on labels is the symbol for catalog number.

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