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CTS™ Dynabeads™ CD3/CD28

Catalog no. 40203D **Store at 2˚C to 8˚C**

Publication No. MAN0008945 **Rev.** F.0 (9 September 2021)

Product contents

Cat. No.	Volume
CTS™ Dynabeads™ CD3/CD28	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).

IMPORTANT: Store CTS™ Dynabeads™ CD3/CD28 tube upright to keep beads in suspension.

Product description

CTS™ Dynabeads™ CD3/CD28 are intended for isolation, activation, and *ex vivo* expansion (1–9) of human T cells for cell-based clinical research.

CTS™ Dynabeads™ CD3/CD28 are produced according to cGMP (21 CFR Part 820, ISO 13485 and ISO 9001) in a qualified ISO 5 (100) clean room. Sterility and endotoxin tests are performed according to current United States Pharmacopeia (USP).

Required materials not supplied

The materials and equipment in the following list are recommended for use with CTS™ Dynabeads™ CD3/CD28 procedures. Alternative materials and equipment may be used.

- CTS™ DynaMag™ Magnet (recommended for larger volumes where the PBMC and CTS™ Dynabeads™ CD3/CD28 beads are in closed bags. See manual for examples of T cell isolation and activation using single-use closed-systems).
  See **thermofisher.com/magnets** for additional recommendations
- CTS™ DPBS (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)
- CTS™ IL-2 Recombinant Human Protein
- Single use separation bags for magnetic isolation (1-L Isolation Bags) and cell expansion (1–3 L Cell Culture Bags), including connectors, filters, and clamps
- Flow cytometer and flurochrome labeled antibodies (as required)
- Sample mixer allowing tilting, rocking, or rotation at 1–3 rpm
- Plasma thawing device
- Bioreactor

Important guidelines

- Follow universal precautions when working with human serum, plasma, or blood products.
- Gloves and a laboratory coat must be worn when working with human samples.
- 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.
- All human samples must be treated as a potential source of HIV, HBV, and other bloodborne pathogens.
- Materials contaminated with blood products must be decontaminated by an approved chemical method and disposed of in labeled biohazard containers.

Prepare media

Prepare fresh media as directed in the table.

Isolation Medium	Expansion Medium
CTS™ DPBS Ca^{2+} and Mg^{2+} free, with 1% HSA or CTS™ OpTmizer™ T-Cell Expansion SFM (see Technical note 1)	CTS™ OpTmizer™ T-Cell Expansion SFM with 2–5% CTS™ Immune Cell SR and 200 IU/mL of CTS™ IL-2 Recombinant Human Protein

Protocol for large scale isolation, activation and expansion of $CD3^+$ T cells

This protocol describes isolation, activation, and expansion of human T cells with cryopreserved samples from apheresis.

- Cultures may also be initiated with fresh samples from apheresis, such as Ficoll separated whole blood, cord blood, bone marrow, or the lymphocyte fraction of elutriated apheresis products.
- The protocol for polyclonal $CD3^+$ T cells uses a ratio of three CTS™ Dynabeads™ CD3/CD28 beads for each T cell.

Thaw and wash cryopreserved cells

Thaw and wash cryopreserved cells according to the internal procedures used at your facility.

Determine the number of $CD3^+$ T cells

Remove 1 mL of washed cells to calculate the viability, concentration, and number of $CD3^+$ T cells.

Isolate and activate $CD3^+$ T cells

The volumes given are intended for isolation of 5 × 10^8 T cells. Adjust the volumes accordingly when using lower/higher cell numbers based on the examples given in Table 1.

1. Resuspend 5 × 10^8 $CD3^+$ T cells in 50–60 mL Isolation Medium (see Technical note 1), and add the resuspended cells to the Isolation Bag.
2. Wash CTS™ Dynabeads™ CD3/CD28 magnetic beads (in tube).
   Note: Alternatively, the CTS™ Dynabeads™ CD3/CD28 can be washed directly in the isolation bag prior to the addition of cells (see Technical note 2).
   a. Resuspend the CTS™ Dynabeads™ CD3/CD28 by vortexing the vial for >30 sec, or tilt and rotate for 30 min.
   b. Transfer 3.75 mL of CTS™ Dynabeads™ CD3/CD28 to a tube.
   c. Add 3.75 mL of Isolation Medium and mix by vortexing.
   d. Place the tube in a DynaMag™-15 magnet for 1 min and discard the supernatant.
   e. Remove the tube from the DynaMag™-15 magnet, then resuspend the washed magnetic beads in 3.75 mL of Isolation Medium.
3. Add the washed CTS™ Dynabeads™ CD3/CD28 to the Isolation Bag containing the resuspended cells from step 1.
4. Add 100 mL of air to a 1-L Isolation Bag.
5. Place the Isolation Bag on a sample mixer and mix at 1–3 rpm for 30 min at room temperature to gently mix the cells and CTS™ Dynabeads™ CD3/CD28 (see Technical note 1).
6. After mixing, remove the Isolation Bag from the mixer. Handle the Isolation Bag gently to avoid disrupting the CTS™ Dynabeads™ CD3/CD28/cell complexes.
7. Drain 150 mL of Isolation Medium into the Isolation Bag.
8. Place the Isolation Bag directly on the CTS™ DynaMag™ Magnet at a –15˚ angle, and incubate the Isolation Bag for 1 min to capture the CTS™ Dynabeads™ CD3/CD28 bound CD3+ T cells.
9. Adjust the magnet to a 60˚ angle.
10. Drain waste fluid containing unbound cells into a Waste Bag (>300 mL capacity) by gravity flow.
11. Immediately remove the Isolation Bag containing the captured cells from the magnet, and add 200 mL Expansion Medium to the Isolation Bag, then gently resuspend the cell/bead complexes.
12. Transfer the media containing cell/CTS™ Dynabeads™ CD3/CD28 complexes from the Isolation Bag to a 1-L CO_2 permeable Cell Culture Bag.
13. Wash the Isolation Bag with 100 mL Expansion Medium and transfer the media to the Cell Culture Bag.
14. Place the Cell Culture Bag in an incubator at 37˚C/5% CO_2 and allow it to remain undisturbed until day 3 of culture.
15. Collect a sample of the non-captured cell fraction and count the number of non-captured cells.
16. Determine the number of CD3+ T cells in the starting fraction and in the non-captured fraction to calculate the isolation efficacy.

Expand $CD3^+$ T cells

The following procedure describes expansion of $CD3^+$ T cells in static Cell Culture Bags. Expansion can also be performed in a bioreactor according to the manufacturer's instructions (see Technical note 3–5).

1. Evaluate the cell concentration at day 3 of culture.
   a. Resuspend the cells by mixing the bag gently to dissociate cell/bead complexes.
   b. Remove 1–2 mL cell suspension sample for counting.
2. Count the number of T cells and adjust the cell concentration to 0.5 × 10^6 cells/mL in Complete Medium. Split the culture into new 1 to 3-L Cell Culture Bags as needed.
3. Repeat counting of cells and expand cells as required for your application.

Harvest expanded $CD3^+$ T cells

1. Harvest cells on an optimal day for your application (usually day 6–12).
2. Remove the Cell Culture Bags from the incubator. Remove a sample from a representative number of bags for cell count and FACS analysis.
3. Remove the CTS™ Dynabeads™ CD3/CD28 by passing the cell culture over the CTS™ DynaMag™ Magnet as described in the CTS™ DynaMag™ Magnet User Guide.
4. Concentrate the cells and wash using a cell washer.
5. Determine the number of residual CTS™ Dynabeads™ CD3/CD28 as described in reference 10 and "Determination of residual Dynabeads™ magnetic beads in the bead-free cell suspension" in the CTS™ DynaMag™ Magnet User Guide, or by digital imaging.

Technical notes

[1] If the starting sample contains less than 25% $CD3^+$ T cells it may be beneficial to mix the sample for 1–2 hours in CTS™ OpTmizer™ T-Cell Expansion SFM instead of CTS™ DPBS Ca^{2+} and Mg^{2+} free, with 1% HSA.

[2] To wash CTS™ Dynabeads™ CD3/CD28 in bag, transfer CTS™ Dynabeads™ CD3/CD28 to a bag. Sterile transfer of CTS™ Dynabeads™ CD3/CD28 from the original vial to a bag can be achieved using a 20 mm vial adapter in combination with a luer-lock syringe and 3-way stopcock. Add the same volume Isolation Medium to the bag. Agitate the bag to ensure mixing of CTS™ Dynabeads™ CD3/CD28 and media. Remove the media from the bag following the same procedure as for magnetic isolation of

Table 1: Volumes for "Isolate and activate $CD3^+$ T cells"

StartingT cell number	Step 1	Step 2	Step 3b	Step 7	Step 12	Step 12	Step 14	Step 15
	Isolation bag size	Starting T cell volume	Bead volume	Isolation Medium volume	Expansion Medium volume	Cell culture bag size	Expansion Medium volume	Final volume in culture bag
5 × 10^8	1000 mL	50 mL	3.75 mL	150 mL	200 mL	1 L	100 mL	300 mL
1 × 10^9	1000 mL	100 mL	7.5 mL	150 mL	200 mL	1 L	400 mL	600 mL

T cells using the CTS™ DynaMag™ Magnet, and resuspend the beads to the desired concentration by adding new Isolation Medium. CTS™ Dynabeads™ CD3/CD28 can either be washed in a dedicated bag or in the same bag that will be used for incubation with cells.

[3] Using a bioreactor (e.g., Xuri™ Cell Expansion System), increases expansion efficiency via perfusion and improved aeration (rocking). Where typical cell densities rarely exceed 2–3 × 10^6 T cells/mL in static cultures, bioreactor systems can readily maintain viable T cells at densities of 2–4 × 10^7 T cells/mL.

[4] To increase the expansion rate, add 2–5% CTS™ Immune Cell SR (19) or human AB serum. Adding a radical scavenger, such as N-acetylcysteine (NAC, 10 mM final concentration) may increase expansion (16). IL-2 can be exchanged in complete medium with alternative common gamma chain cytokines such as IL-7 or IL-15 to better preserve a stem central memory/central memory T cell subset *ex vivo* (11–12).

[5] 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) (13). 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.

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. Day 1, 2, and/or 3 are recommended as optimal days for transduction using lentivirus-based vectors. 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 is acceptable for most transduction applications.

General Information

Certification

Life Technologies AS complies with Quality System Standard ISO 13485:2016EN ISO 13485:2016

"Design, development and manufacturing and distribution of *in vitro* diagnostic assay components, products intended for *ex-vivo* separation of human cells, and for cell-based clinical research and of reagents used for life science applications."

CTS™ Dynabeads™ CD3/CD28 is manufactured at Thermo Fisher Scientific Baltics UAB complying with ISO 13485:2016EN ISO 13485:2016 with the scope" Design, development and manufacturing of life science products, including proteins, nucleic acids, nucleotides, antibodies and associated kits, for research and *in vitro* and *in vivo* diagnostics, as well as manufacturing of the materials intended for *ex-vivo* separation of human cells and for cell-based clinical diagnostics and for therapeutic applications, including processes under aseptic conditions.

In the United States, CTS™ Dynabeads™ CD3/CD28 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 LoARequestCTSDynabeads@thermofisher.com with the sponsor's and/or investigator's full name and address, along with project name and aim. This information is required by Thermo Fisher Scientific 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

CTS™ Dynabeads™ CD3/CD28 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.
CTS™ DynaMag™ Magnet	12102
DynaMag™-15 Magnet	12301D
CTS™ DPBS without calcium chloride, without magnesium chloride	A1285601
CTS™ Immune Cell SR 50 mL 500 mL	 A2596101 A2596102
CTS™ OpTmizer™ T-Cell Expansion 1000 mL (bottle) 1000 mL (bag)	 A1048501 A1048503
CTS™ OpTmizer™ T-Cell Expansion, no phenol red 1000 mL (bottle) 1000 mL (bag)	 A3705001 A3705003
CTS™ IL-2 Recombinant Human Protein	CTP0021 or CTP0023

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Ex vivo activation or expansion of human T-cells

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