CTS™ Detachable Dynabeads™ CD3/CD28 and CTS™ Detachable Dynabeads™ Release Buffer (Manual Workflow)

Catalog Numbers A56996, A5588301, A5588302, A5588303

Pub. No. MAN1000166 Rev. A

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ CTS™ Detachable Dynabeads™ CD3/CD28 simultaneously isolates and activates human T cells based on CD3 and CD28 co-expression. When used with the CTS™ Detachable Dynabeads™ Release Buffer, users are able to actively released the CTS™ Detachable Dynabeads™ magnetic beads at any time point, depending on the user's desired process. Instead of relying on passive dissociation of the magnetic beads, the active release technology is critical to i) control activation time, ii) release beads prior to T cell manufacturing steps where the presence of beads is unwanted, and iii) to enable shortening of T cell manufacturing process to a few days.

This user guide provides a test protocol for a small-scale, manual process for isolation, activation, and release of human T cells. For clinical T cell manufacturing, see *CTS™ Detachable Dynabeads™ CD3/CD28 and CTS™ Detachable Dynabeads™ Release Buffer (Automated Workflow) User Guide* (Pub. No. MAN1000167) for a fully automated large-scale workflow.

Contents and storage

Table 1 Usage and storage for CTS™ Detachable Dynabeads™ CD3/CD28 and CTS™ Detachable Dynabeads™ Release Buffer

Product	Cat. No.	Volume	Capacity	Storage
CTS™ Detachable Dynabeads™ CD3/CD28	A56996	10 mL	Can isolate and activate (in one step) up to 1.3×10^9 T cells.	5 ± 3°C; Store vial upright to keep beads in suspension.
CTS™ Detachable Dynabeads™ Release Buffer	A5588303	212 mL	For use with up to three (3) CTS™ Detachable Dynabeads™ vials.	5 ± 3°C; Protected from light.
	A5588301	400 mL	For use with up to six (6) CTS™ Detachable Dynabeads™ vials.	
	A5588302	750 mL	For use with up to twelve (12) CTS™ Detachable Dynabeads™ vials.	

Table 2 Contents for CTS™ Detachable Dynabeads™ CD3/CD28 and CTS™ Detachable Dynabeads™ Release Buffer

Product	Contents
CTS™ Detachable Dynabeads™ CD3/CD28	4×10^8 beads/mL in phosphate buffered saline (PBS), pH 7.4, with 0.1% recombinant human albumin and 0.01% Tween™ 80 detergent
CTS™ Detachable Dynabeads™ Release Buffer[1]	DPBS with 5 mM biotin and 0.5% recombinant human albumin, pH 7.2

[1] This product may develop minor protein aggregates after agitation, however, this does not affect product quality or performance.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Table 3 Required material not supplied

Item	Source
Reagents	
CTS™ DPBS without calcium chloride, without magnesium chloride	A1285601
Recombinant human albumin (rHA) or Human serum albumin (HSA)	MLS
CTS™ OpTmizer™ T-Cell Expansion SFM (Serum Free Medium) with or without phenol red	A1048501, A3705001
CTS™ Immune Cell SR (Serum Replacement)	A2596101
CTS™CTS™ IL-2 Recombinant Human Protein or other cytokines according to user specific protocol	CTP0021, CTP0023
L-Glutamine	MLS
Equipment	
DynaMag™-5 Magnet, DynaMag™-15 Magnet, DynaMag™-50 Magnet	12303D, 12301D, 12302D
Flow cytometer and fluorochrome labeled antibodies as required	MLS
Vortex mixer	MLS
Sample mixer allowing gentle tilting, rocking, or rotation	MLS
Cell culture vessel (e.g. plate, flask)	MLS
Laminar air flow unit	MLS
CO_2 Incubator	MLS

Generic protocol

1. For optimal isolation and activation in one step, use a ratio of three beads per $CD3^+$ T cell, and a T cell concentration of 1×10^7 cells/mL in DPBS with 1% rHA/HSA.
2. Incubate for 30 minutes with gentle tilting and rotation.

   Bead-bound T cells are retained on a magnet and unbound cells can be washed away.
3. Carefully resuspend bead-bound T cells in culture media and activate in humidified atmosphere of 5% CO_2 in air at a temperature of 37°C for the desired time, typically 1 to 3 days.
4. Terminate T cell activation signaling by incubating for 60 minutes with the CTS™ Detachable Dynabeads™ Release Buffer, which actively detaches the beads from the T cells.
5. Use a DynaMag™ magnet to remove beads.

   Released cells are bead-free and ready for downstream gene-modification and expansion.

Protocol for manual small-scale isolation and activation of up to 5×10^7 T cells

1. Resuspend Peripheral blood mononuclear cells (PBMCs) in DPBS with 1% rHA/HSA at a cell concentration of 1×10^7 $CD3^+$ T cells/mL. For examples of tubes and volumes see Table 4.
2. Calculate the amount of CTS™ Detachable Dynabeads™ CD3/CD28 needed based on a recommended ratio of 3 beads per $CD3^+$ T cell.
3. Resuspend the CTS™ Detachable Dynabeads™ CD3/CD28 by vortexing the vial for > 5 seconds. Then tilt and rotate for 15 minutes (Table 4).

4. Wash the CTS™ Detachable Dynabeads™ CD3/CD28 in a tube.
    a. Immediately after resuspension of beads, transfer the volume calculated in step 2 into a tube and place on a DynaMag™ magnet. See Table 4.
    b. Leave for 1 minute and remove supernatant.
    c. Immediately, add 5–10x bead suspension volume of DPBS with 1% rHA/HSA.
    d. Mix by vortexing 5 seconds and place on magnet.
    e. Leave for 1 minute and remove washing buffer.
    f. Resuspend beads in the same volume as originally added of bead suspension from step 2.
5. Add the washed CTS™ Detachable Dynabeads™ CD3/CD28 to the cell suspension in step 1.
6. Incubate for 30 minutes with gentle tilting and rotation at room temperature.
7. Place tube in a DynaMag™ magnet. Leave for 1 minute and transfer the supernatant with unbound cells to a new tube.
8. Immediately wash the bead bound cells to improve purity of isolated cells. Add the same volume as cell suspension in step 1 of DPBS with 1% rHA/HSA to the tube, mix by 5 seconds vortexing.
9. Place tube in a DynaMag™ magnet. Leave for 1 minute and transfer the supernatant to tube in step 7.
10. Repeat step 8 and step 9.
11. Immediately, carefully resuspend the bead-bound cells by adding a small volume of culture media and gentle tilting.
12. Transfer the bead-bound cells to a cell culture vessel, and add culture media to desired T cell concentration, typically 0.5×10^6 –1×10^6 cells/mL.
13. Activate T cells for the desired time, typically 1–3 days in a CO_2 incubator at 37°C.

    Note: A variety of protocols may be used for T cell expansion in different culture vessels. Optimal procedures should be determined empirically by the investigator. Feed and maintain cells at desired concentrations while cells are in log phase growth. To maintain log phase growth in static cultures, it may be preferable to split cells to achieve a density of 0.5–1×10^6 cells/mL.

Protocol for manual small-scale release of the CTS™ Detachable Dynabeads™ CD3/CD28 from isolated and activated T cells

1. Transfer the culture containing beads and T cells to tube(s) and place on a DynaMag™ magnet (see Table 4). Leave for 1 minute.
2. Transfer the supernatant to a new tube(s) and keep, as supernatant may contain spontaneously released T cells.
3. Immediately add CTS™ Detachable Dynabeads™ Release Buffer to the tube(s) containing bead bound T cells (See Table 4).
4. Incubate for 60 minutes with gentle tilting and rotation at room temperature.
5. Resuspend CTS™ Detachable Dynabeads™ CD3/CD28 and cells by 10x pipetting.
6. Place tube in a DynaMag™ magnet. Leave for 1 minute and transfer the supernatant with the released cells to the tube with the supernatant from step 2.
7. To increase the yield, wash the bead fraction by adding same volume as in step 1 with DPBS with 1% rHA/HSA and vortex for 5 seconds to mix.
8. Place tube(s) in a DynaMag™ magnet. Leave for 1 minute and transfer the supernatant to the same tube as in step 6.
9. Repeat step 7 and step 8 twice.
10. Remove CTS™ Detachable Dynabeads™ Release Buffer by centrifugation at 350 × *g* for 5–10 minutes.
11. Resuspend T cells in culture media and repeat centrifugation step.

12. Resuspend T cells in culture media for downstream expansion or buffer for gene-modification.

Table 4 Suggested volumes and equipment for isolation and activation of T cells (manual workflow)

Starting number of $CD3^+$ T cells and volume	CTS™ Detachable Dynabeads™ CD3/CD28 number and volume[1]	Test tubes for isolation and release	DynaMag™ magnet	CTS™ Detachable Dynabeads™ Release Buffer
1×10^7 $CD3^+$ T cells in 1 mL	3×10^7 beads in 75 µL	3.6 mL Nunc™ tube (isolation) and 15 mL Nunc™ tubes (release and wash)	DynaMag™-5 Magnet and DynaMag™-15 Magnet	2 mL
5×10^7 $CD3^+$ T cells in 5 mL	15×10^7 beads in 375 µL	15 mL Nunc™ tube (isolation) and 50 mL Nunc™ tubes (release and wash)	DynaMag™-15 Magnet and DynaMag™-50 Magnet	10 mL

[1] Dynabeads™ magnetic beads must be resuspended before use to make a homogenous solution.

Technical notes

If release is performed on day 1, DNA-caused aggregates can trap cells and reduce yield. A short incubation with DNase (30 U/mL, 1–5 mins) before placing the tube in the DynaMag™ magnet will dissolve aggregates and increase the yield in the upcoming release step.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A	15 May 2024	New document for CTS™ Detachable Dynabeads™ CD3/CD28 and CTS™ Detachable Dynabeads™ Release Buffer (Manual Workflow).