

CTS™ Detachable Dynabeads™ CD4, CTS™ Detachable Dynabeads™ CD8, CTS™ Detachable Dynabeads™ Release Buffer (Manual Workflow)

Catalog Numbers A56994, A56995, A5588303, A5588301, A5588302

Pub. No. MAN1000203 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](https://www.thermofisher.com/support).

Product description

Gibco™ CTS™ Detachable Dynabeads™ CD4 and CTS™ Detachable Dynabeads™ CD8 positively isolate human T cell subsets. These CTS™ Detachable Dynabeads™ magnetic beads are intended to be used with the CTS™ Detachable Dynabeads™ Release Buffer that enables active release of magnetic beads at any time. This results in bead-free T cells for downstream processes such as gene-editing and activation.

This user guide provides a test protocol for a small-scale, manual process for isolation and active release of human T cells using the CTS™ Detachable Dynabeads™ magnetic beads. For clinical T cell manufacturing, see *CTS™ Detachable Dynabeads™ CD4, CTS™ Detachable Dynabeads™ CD8, CTS™ Detachable Dynabeads™ Release Buffer (Automated Workflow) User Guide* (Pub. No. [MAN1000204](#)) for a fully automated large-scale workflow. The manual protocol will be identical independent of using CTS™ Detachable Dynabeads™ CD4 or CTS™ Detachable Dynabeads™ CD8, individually, or a combination of the two CTS™ Detachable Dynabeads™ magnetic beads together to isolate CD4⁺ and CD8⁺ T cells.

Contents and storage

Table 1 Usage and storage for CTS™ Detachable Dynabeads™ (CD4 or CD8) and CTS™ Detachable Dynabeads™ Release Buffer

Product	Cat. No.	Volume	Capacity	Storage
CTS™ Detachable Dynabeads™ CD4	A56994	15 mL	Can isolate and release up to 1.5×10^9 CD4 ⁺ T cells.	5 ± 3°C; Store vial upright to keep beads in suspension.
CTS™ Detachable Dynabeads™ CD8	A56995	15 mL	Can isolate and release up to 1.5×10^9 CD8 ⁺ T cells.	
CTS™ Detachable Dynabeads™ Release Buffer	A5588303	212 mL	For use with up to 30 mL of CTS™ Detachable Dynabeads™ beads.	5 ± 3°C; Protected from light.
	A5588301	400 mL	For use with up to 60 mL of CTS™ Detachable Dynabeads™ beads.	
	A5588302	750 mL	For use with up to 120 mL of CTS™ Detachable Dynabeads™ beads.	

Table 2 Contents of CTS™ Detachable Dynabeads™ (CD4 or CD8) and CTS™ Detachable Dynabeads™ Release Buffer

Product	Contents
CTS™ Detachable Dynabeads™ (CD4 or CD8)	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](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier.

Table 3 Required materials not supplied

Item	Source
Reagents	
CTS™ DPBS without calcium chloride, without magnesium chloride	A1285601
Recombinant human albumin (rHA) or Human serum albumin (HSA)	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

Generic protocol

Target cells = CD4⁺ and/or CD8⁺ cells

- For optimal isolation, use a ratio of four beads per target cell, and a target cell concentration of 1×10^7 target cells/mL in DPBS with 1% rHA/HSA for isolation.
- Incubate for 10 minutes with gentle tilting and rotation.
Bead-bound T cells are retained on a magnet and unbound cells can be washed away.
- Incubate for 60 minutes with the CTS™ Detachable Dynabeads™ Release Buffer, which actively detaches the beads from the T cells.
- 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

- Resuspend peripheral blood mononuclear cells (PBMCs) in DPBS with 1% rHA/HSA at a cell concentration of 1×10^7 target cells/mL. For examples of tubes and volumes see Table 4.
Note: If desired to isolate all T cells using a combination of the CTS™ Detachable Dynabeads™ CD4 and CTS™ Detachable Dynabeads™ CD8, considering the probable skewed ratio of target cells: adjust cell concentration to 1×10^7 CD3⁺ cells.
- Calculate the amount of CTS™ Detachable Dynabeads™ magnetic beads needed based on a recommended ratio of 4 beads per target cell.
- Resuspend the magnetic beads by vortexing the vial for > 5 seconds. Then gently tilt and rotate for 15 minutes.
- Wash the magnetic beads in a tube by following substep 4a to substep 4f.
 - Immediately after resuspension of beads, transfer the volume calculated in step 2 into a tube and place on a DynaMag™ magnet. See Table 4.
 - Leave for 1 minute and remove supernatant.
 - Immediately, add 5–10x bead suspension volume of DPBS with 1% rHA/HSA.
 - Mix by vortexing 5 seconds and place on magnet.
 - Leave for 1 minute and remove washing buffer.
 - Resuspend beads in the same volume as originally added of bead suspension from step 2.
- Add the washed magnetic beads to the cell suspension in step 1.
- Incubate for 10 minutes with gentle tilting and rotation at room temperature.

- Place tube in a DynaMag™ magnet. Leave for 1 minute and transfer the supernatant with unbound cells to a new tube.
- 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.
- Place tube in a DynaMag™ magnet. Leave for 1 minute and transfer the supernatant to tube in step 7. The tube now contains your non-selected cells.
- Repeat step 8 and step 9. The tube now contains your non isolated cells.
- Immediately add CTS™ Detachable Dynabeads™ Release Buffer to the tube containing bead bound T cells.
- Incubate for 60 minutes with gentle tilting and rotation at room temperature.
- Resuspend magnetic beads and cells by pipetting up and down 10 times.
- Place tube in a DynaMag™ magnet. Leave for 1 minute and transfer the supernatant with the released cells to a new tube.
- To increase the yield, wash the bead fraction by adding same volume as in step 11 with DPBS with 1% rHA/HSA and vortex for 5 seconds to mix.
- Place tube(s) in a DynaMag™ magnet. Leave for 1 minute and transfer the supernatant to the same tube as in step 14.
- Repeat step 15 and step 16 (total of 3 washes). The tube now contains your selected target cells.
- Remove CTS™ Detachable Dynabeads™ Release Buffer from target cells by centrifugation at 350 × g for 5–10 minutes.
- Resuspend T cells in culture media or any desired buffer and repeat centrifugation step.
- Resuspend the target cells in culture media for downstream activation and expansion, or buffer for gene modification.

Table 4 Suggested volumes and equipment for isolation of target cells (manual workflow). Examples below are for single isolation of CD4+ or CD8+ cells.

Starting number of target cells and volume	CTS™ Detachable Dynabeads™ magnetic beads number and volume	Test tubes for isolation and release	DynaMag™ magnet	CTS™ Detachable Dynabeads™ Release Buffer
1 × 10 ⁷ target cells in 1 mL	4 × 10 ⁷ beads (100 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 target cells in 5 mL	20 × 10 ⁷ beads (500 µL)	15 mL Nunc™ tube (isolation) and 50 mL Nunc™ tubes (release and wash)	DynaMag™-5 Magnet and DynaMag™-50 Magnet	10 mL

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	29 August 2024	New document for CTS™ Detachable Dynabeads™ CD4, CTS™ Detachable Dynabeads™ CD8, CTS™ Detachable Dynabeads™ Release Buffer (Manual Workflow)

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