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

Catalog Numbers A56996, A5588301, A5588302, A5588303

Pub. No. MAN1000167 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 release 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.

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.

Table 3 Recommendations for CTS™ Detachable Dynabeads™ CD3/CD28 and CTS™ Detachable Dynabeads™ Release Buffer

Component	Recommendations
CTS™ Detachable Dynabeads™ CD3/CD28	CTS™ Detachable Dynabeads™ CD3/CD28 3:1 bead to target cell ratio (target cell = $CD3^+$). Incubation time with input cells and CTS™ Detachable Dynabeads™ magnetic beads is 30 minutes.
CTS™ Detachable Dynabeads™ Release Buffer	CTS™ Detachable Dynabeads™ Release Buffer 5 mL per 1 mL of CTS™ Detachable Dynabeads™ magnetic beads. Incubation time with CTS™ Detachable Dynabeads™ Release Buffer is 60 minutes.

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 4 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
CTS™ OpTmizer™ T-Cell Expansion SFM (Serum Free Medium)	A3705003
L-Glutamine	MLS
CTS™ Immune Cell SR (Serum Replacement)	A2596101
CTS™ IL-2 Recombinant Human Protein or other cytokines according to user specific protocol	CTP0021, CTP0023
Consumables	
CTS™ DynaCellect™ Cell Isolation Kit	A52300
Cell culture vessel (e.g. plate, flask)	MLS
5 mL luer-lock syringe	15869152
60 mL luer-lock syringe	14955461
Conical bags for beads and/or cell input	OriGen Biomedical; CSD400Y9
Spike to female luer	OriGen Biomedical; S-F50, S-F10
1L blood bag and clamps	Terumo; T100BM
150 mL blood bag and clamps	Terumo; T015CM
Equipment	
CTS™ DynaCellect™ Magnetic Separation System	A55868
Sterile tubing welder	MLS
Tube sealer	MLS
Leukopak thaw system	MLS
Cell counter	MLS
Flow cytometer and fluorochrome labeled antibodies as required	MLS
Vortex mixer	MLS
Sample mixer allowing gentle tilting, rocking, or rotation	MLS
Laminar air flow unit	MLS
CO_2 incubator	MLS

Preparation of the Single-Use Kits

CTS™ DynaCellect™ Magnetic Separation System User Guide (Pub. No. MAN0026480), Chapter 6 (Basic instrument operation) contains a detailed description of the consumables preparation for a run both for isolation and bead removal protocols.

Example protocol for automated isolation and activation of 4 × 10^8 cells in 40 mL

Note: The following is a sample protocol. Users will need to optimize and/or adapt for their specific process.

1. Prepare the consumables according to the configuration shown in Figure 1. For the type of bags, content, and volumes, see Table 5. Place clamps on filled bags.

   Note: Line B and E must be connected to two different ports on the same bag to enable purging of air in the system.

Figure 1 Example consumable configuration of isolation kit for CTS™ Detachable Dynabeads™ CD3/CD28

- A: Isolation
- B: Wash buffer
- C: Output
- D: —
- E: Wash buffer
- F: Cell media
- G: Cell input
- H: CTS™ Detachable Dynabeads™ CD3/CD28
- I: Supplement

Table 5 Bags, content and volumes used for cell isolation configuration

Content	Bag/ Vessel	Tube line	Volume
Wash buffer: DPBS/1% rHA of HSA	1 L Terumo bag	B. E	550 mL
Output	1 L Terumo bag/ closed culture system	C	Empty/ prefilled closed system with the appropriate volume of media
Complete OpTmizer™ cell media	1 L Terumo bag	F	500 mL
Cell input	Conical bag	G	40 mL (10^7 target cells/mL)
CTS™ Detachable Dynabeads™ CD3/CD28 (4 × 10^8 beads/mL)	Conical bag	H	3 mL (3:1 bead to target cell ratio)
Supernatant	1 L Terumo bag	I	Empty

2. Thaw a leukopak. Dilute the content 1:1 with DPBS/1% rHA or HSA to decrease the concentration of DMSO. Measure cell concentration in a cell counter and use the percentage of $CD3^+$ from the Certificate of Analysis (CoA) to adjust the concentration to 10^7 target cells ($CD45^+CD3^+$)/mL. Transfer 40 mL of the cell suspension to the cell input bag (G) using a 60 mL syringe. Add at least 30 mL air into the bag, to trigger the bubble sensor when the bag is empty.

3. Resuspend the CTS™ Detachable Dynabeads™ CD3/CD28 by vortexing the vial for >5 seconds then tilt and rotate for 15 minutes.

4. Transfer 3 mL CTS™ Detachable Dynabeads™ CD3/CD28 to the bead bag (H). Place a large clamp across the top of the bag, to avoid beads being stuck in the top of the bag. Add at least 30 mL air into the bag, to trigger the bubble sensor when the bag is empty.

5. Weld the bags to the CTS™ DynaCellect™ Cell Isolation Kit, following the configuration shown in Figure 1. Hook the bags onto the bag hangers.

   IMPORTANT! After welding, open the weld by rolling the tube and check the weld integrity by pulling on the tubes from each side.

   Note: The point of welding is more susceptible to folding and occluding the liquid path.

6. Install the CTS™ DynaCellect™ Cell Isolation Kit on the instrument following the instructions in chapter 6 (Basic instrument operation) in the *CTS™ DynaCellect™ Magnetic Separation System User Guide* (Pub. No. MAN0026480).

7. Run the appropriate protocol on CTS™ DynaCellect™ Magnetic Separation System. Follow instructions in Chapter 3 (Embedded graphical user interface (eGUI) user instructions) in the *CTS™ DynaCellect™ Magnetic Separation System User Guide* (Pub. No. MAN0026480).

   IMPORTANT! Before starting the run, close pinch valves, remove clamps, and make sure there are no kinks in the tubes.

8. After the isolation protocol is complete, seal off the output line (C), containing the bead-bound cells. Transfer the content into an appropriate culture vessel, if not already in one, and add culture media to desired T cell concentration.

9. Activate T cells for the desired time, typically 1–3 days at 37°C and 5% CO_2.

   Note: If release is performed on Day 1, DNA-caused aggregates can trap cells and reduce yield. A short incubation of the input material (cells attached to beads) with DNase (30 U/mL, 1–5 mins) will dissolve aggregates and increase the yield in the following release step.

Example protocol for automated release of the CTS™ Detachable Dynabeads™ CD3/CD28 from isolated and activated T-cells

1. Prepare the consumables as shown in Figure 2. For the type of bags, content, and volumes see Table 6. Place a clamp on any filled bags.

   Note: Line B and E must be connected to two different ports on the same bag to enable purging of air in the system.

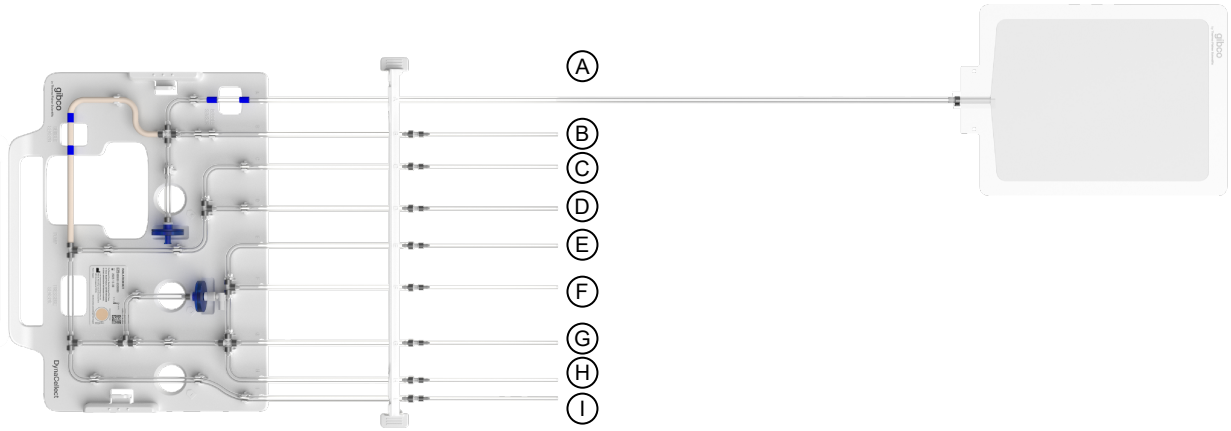

Figure 2 Example consumable configuration of isolation kit for CTS™ Detachable Dynabeads™ CD3/CD28 for release.

- A: Isolation
- B: Wash buffer
- C: Output
- D: —
- E: Wash buffer
- F: Release buffer
- G: Input
- H: —
- I: —

Table 6 Bags, content, and volumes used for the release protocol

Content	Bag/vessel	Tube line	Volume
Wash buffer: DPBS/1% rHA or HSA	1 L Terumo bag	B. E	400 mL
Output	1 L Terumo bag, Grex culture vessel, or other appropriate culture vessel	C	Empty, or prefilled with an appropriate volume of media
CTS™ Detachable Dynabeads™ Release Buffer	150 mL Terumo bag	F	5 mL per mL of [1]
Input	Conical bag	G	200 mL

[1] The recommended amount of CTS™ Detachable Dynabeads™ Release Buffer is 5 mL per mL of CTS™ Detachable Dynabeads™ magnetic beads. If the DynaCellect™ protocol is programmed to drain the release buffer bag (bubble sensor activated), the exact volume of required release buffer should be in the bag. However, if the DynaCellect™ protocol is programmed to pump a set volume of release buffer, 20% additional volume of CTS™ Detachable Dynabeads™ Release Buffer is recommended to avoid introducing air into the system. Also, it is recommended to account for dead volume within the CTS™ DynaCellect™ Cell Isolation Kit

2. Concentrate the bead-bound cells to 200 mL final volume by removing the supernatant from the cell culture, transfer the bead-bound cells suspension to the input bag (G) using a 60 mL syringe. Add at least 30 mL air into the bag, to trigger the bubble sensor when the bag is empty.

IMPORTANT! Handle the cell culture with care when concentrating, such that the cells do not go into the supernatant.

3. Fill release buffer bag (F) with 37 mL CTS™ Detachable Dynabeads™ Release Buffer.

   Note: 37 mL CTS™ Detachable Dynabeads™ Release Buffer volume results from the following calculations: 5 mL per mL of CTS™ Detachable Dynabeads™ magnetic beads = 5 × 3 = 15, +16 mL dead volume from F to A lines , +20% extra CTS™ Detachable Dynabeads™ Release Buffer = 15 + 16 = 31, 20% of 31 = 6.2 mL

4. Weld the bags to the CTS™ DynaCellect™ Cell Isolation Kit, following the configuration shown in Figure 2. Hook the bags onto the bag hangers.

IMPORTANT! After welding, open the weld by rolling the tube and check the weld integrity by pulling on the tubes from each side. Note that the point of welding is more susceptible to folding and occluding the liquid path.

5. Install the CTS™ DynaCellect™ Cell Isolation Kit on the instrument following the instructions in chapter 6 (Basic instrument operation) in the *CTS™ DynaCellect™ Magnetic Separation System User Guide*.

6. Run the appropriate release protocol on CTS™ DynaCellect™ Magnetic Separation System. Follow instructions in Chapter 3 (Embedded graphical user interface (eGUI) user instructions) in the *CTS™ DynaCellect™ Magnetic Separation System User Guide* (Pub. No. MAN0026480).

IMPORTANT! Before starting the run, close pinch valves, remove clamps, and make sure there are no kinks in the tubes, or otherwise occluded fluid paths.

7. After the protocol is complete, seal off the output line (C), containing the released cells in CTS™ Detachable Dynabeads™ Release Buffer.

8. Use a closed cell processing device, like the CTS™ Rotea™ Counterflow Centrifugation System (refer to Rotea™ method) to exchange CTS™ Detachable Dynabeads™ Release Buffer with the appropriate buffer or media for downstream applications.

Automated bead removal

If a final clean-up of beads is needed for the manufacturing process, perform bead removal according to *CTS™ DynaCellect™ Magnetic Separation System User Guide* (Pub. No. MAN0026480), Chapter 6 (Basic instrument operation).

Limited product warranty

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

Revision history: Pub. No. MAN1000167 A

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

The information in this guide is subject to change without notice.