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

Catalog Numbers A56994, A56995, A5588301, A5588302, A5588303

Pub. No. MAN1000204 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™ 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 CTS™ Detachable Dynabeads™ magnetic beads at any time, resulting in bead-free T cells for downstream processes such as gene-editing and activation. These products are compatible with the CTS™ DynaCellect™ Magnetic Separation System which automates the isolation, active release, and magnetic separation steps within the cell therapy manufacturing process.

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.

General recommendations

Table 3 Recommendations for CTS™ Detachable Dynabeads™ (CD4 or CD8) and CTS™ Detachable Dynabeads™ Release Buffer

Component	Recommendations
CTS™ Detachable Dynabeads™ (CD4 or CD8)	4:1 bead to target cell ratio (target cell = $CD4^+$ or $CD8^+$). Incubation time with input cells and CTS™ Detachable Dynabeads™ magnetic beads is 10 minutes
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 30 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
Consumables	
CTS™ DynaCellect™ Cell Isolation Kit	A52300
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
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
Biosafety cabinet	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.

Isolate CD4+ and/or CD8+ cells using CTS™ Detachable Dynabeads™ CD4 and/or CTS™ Detachable Dynabeads™ CD8

Example protocol for automated isolation and release of target cells from a ¼ leukopak

Note: The following is a sample protocol. Users will need to optimize and/or adapt for their specific process. See the Protocol optimization section for additional information.

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 bags that will be filled.

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

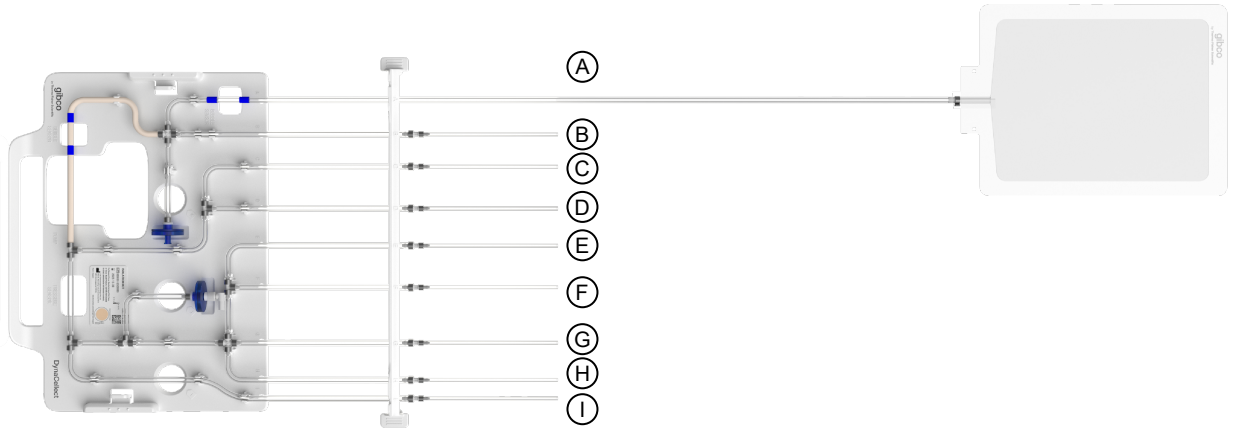

Figure 1 Example consumable configuration of isolation and release kit for CTS™ Detachable Dynabeads™ (CD4 and/or CD8) using a CTS™ DynaCellect™ Cell Isolation Kit.

- A: Isolation bag
- B: Wash buffer
- C: Output
- D: (Line D is not used for this setup)
- E: Wash buffer
- F: CTS™ Detachable Dynabeads™ Release Buffer
- G: Cell input
- H: CTS™ Detachable Dynabeads™ (CD4 and/or CD8)
- I: Supernatant

Table 5 Bags, content and volumes used for CD4 and/or CD8 cell isolation and release configuration

Content	Bag	Tube line	Volume
Wash buffer: DPBS/1% rHA or HSA	1 L Terumo bag	B. E	650 mL
Output	1 L Terumo bag	C	Empty
CTS™ Detachable Dynabeads™ Release Buffer	CTS™ Detachable Dynabeads™ Release Buffer bag	F	5 mL per 1 mL of CTS™ Detachable Dynabeads™ beads.[1]
Cell input	Conical bag	G	¼ leukopak (diluted 1:1 in DPBS/1% rHA or HSA)
CTS™ Detachable Dynabeads™ (CD4 and/or CD8)	Conical bag	H	Dependent on number of CD4+ and/or CD8+ input cells and 4:1 bead to target cell ratio
Supernatant	1 L Terumo bag	I	Empty

[1] The recommended amount of CTS™ Detachable Dynabeads™ Release Buffer is 5 mL per 1 mL of CTS™ Detachable Dynabeads™ 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 DynaCellect™ kit. See section “Protocol optimization” for more details.

2. Thaw the leukopak material. Dilute the content 1:1 with DPBS/1% rHA or HSA to decrease the concentration of cryopreservation media. Measure cell concentration using a cell counter and use the percentage of $CD4^+$ and/or $CD8^+$ from the leukopak Certificate of Analysis (CoA) to calculate the number of target cells. Use the number of target cells to calculate the volume of CTS™ Detachable Dynabeads™ magnetic beads needed, given the recommended 4:1 bead to target cell ratio.

   Example: In a ¼ leukopak, there is 9×10^8 $CD4^+$ and 4×10^8 $CD8^+$ cells (calculated based on cell counts and percentage of target cells from the leukopak CoA). Given the recommended 4:1 bead to target cell ratio, the total number of CTS™ Detachable Dynabeads™ magnetic beads needed is calculated as follows:

   Total number of magnetic beads = 4x number of total target cells

   The total number of CTS™ Detachable Dynabeads™ magnetic beads must be divided by the CTS™ Detachable Dynabeads™ magnetic beads stock concentration to get the required volume of CTS™ Detachable Dynabeads™ magnetic beads.

$$\text{Volume of magnetic beads} = \frac{\text{Total number of magnetic beads}}{\text{Stock concentration of magnetic beads}}$$

   Based on the amount of $CD4^+$ and $CD8^+$ target cells given in this example, the calculation of the volumes of CTS™ Detachable Dynabeads™ magnetic beads required is as follows:

$$\text{Volume of magnetic beads CD4} = \frac{4 \times (9 \times 10^8) \text{ magnetic beads}}{4 \times 10^8 \text{ magnetic beads/mL}} = 9 \text{ mL}$$

$$\text{Volume of magnetic beads CD8} = \frac{4 \times (4 \times 10^8) \text{ magnetic beads}}{4 \times 10^8 \text{ magnetic beads/mL}} = 4 \text{ mL}$$

   Based on these calculations, the volume of CTS™ Detachable Dynabeads™ magnetic beads needed in this example is thus 9 mL of CTS™ Detachable Dynabeads™ CD4 and 4 mL of CTS™ Detachable Dynabeads™ CD8 for a combined isolation. If single isolation is conducted, either 9 mL CTS™ Detachable Dynabeads™ CD4 or 4 mL CTS™ Detachable Dynabeads™ CD8 is required depending on the desired target cell.

3. Place a large clamp across the top of the cell input bag (G), to avoid cells being stuck in the top of the bag. Transfer the cell suspension to the cell input bag using a 60 mL luer lock syringe. Add at least 30 mL air into the bag, to trigger the bubble sensor when the bag is empty.

4. Resuspend the CTS™ Detachable Dynabeads™ CD4 and/or CTS™ Detachable Dynabeads™ CD8 by vortexing the vial for >5 seconds then tilt and rotate for 15 minutes.

5. Place a large clamp across the top of the bead bag (H), to avoid beads being stuck in the top of the bag. Transfer the required amount of CTS™ Detachable Dynabeads™ CD4 and/or CTS™ Detachable Dynabeads™ CD8 to the bead bag. Add at least 30 mL of air into the bag to trigger the bubble sensor when the bag is empty.

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

7. 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).

8. 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.

9. After the isolation and release protocol is complete, seal off the output line (C) containing the target cells.

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

Troubleshooting

Observation	Possible cause	Recommended action
Isolation efficiency (the capacity of CTS™ Detachable Dynabeads™ to isolate target cells) is lower than desired	Target cell concentration is too low.	Adjust input target cell concentration to 10^7 target cells per mL. To achieve this, measure cell concentration using a cell counter and use the percentage of $CD4^+$ or $CD8^+$ from the leukopak CoA to adjust the concentration to 10^7 target cells/mL ($CD4^+$ or $CD8^+$). For a combined isolation of $CD4^+$ and $CD8^+$ cells, adjust the target cell concentration to 10^7 total T cells per mL.
	If the washing after isolation, before releasing the target cells from the CTS™ Detachable Dynabeads™, is too rough, target cells may be washed away together with non-target cells to the supernatant bag.	Adjust the DynaCellect™ protocol to obtain a gentler wash. Changes can be to reduce the number of washes, reduce the volume of wash buffer in each wash or reduce the rocking speed during wash.
Low purity of target cell fraction	Insufficient washing of cells after isolation, before releasing target cells from CTS™ Detachable Dynabeads™ magnetic beads.	Purity of the target cell fraction can be improved by increasing the wash buffer volume and/or number of washes after isolation of target cells, before the target cells are released from the CTS™ Detachable Dynabeads™ magnetic beads.
Low recovery of target cell fraction	Target cells may be retained in the CTS™ DynaCellect™ Cell Isolation Kit.	If experiencing low recovery of target cells, count all fractions to identify where the target cells are. If a high amount of target cells is in the supernatant fraction with the non-target cells, follow the instructions given above for improving isolation efficiency. If the target cells are left with the beads, inspect if they are bead-bound or not. If the cells are bead-bound, ensure that the recommended incubation time and recommended volume of CTS™ Detachable Dynabeads™ Release Buffer is used in the protocol (Table 3). If the cells are not bead-bound, increase the number of wash cycles and/or the volume in each wash cycle (after the cells have been released from the CTS™ Detachable Dynabeads™ magnetic beads) to increase the number of target cells transferred to the output bag.

Protocol optimization

- For further automation of the process, the DynaCellect™ protocol can be programmed to dilute the leukopak 1:1 with wash buffer.
- Transfer of liquid using the DynaCellect™ system can be controlled in different ways. Either by transferring a set volume (bubble sensor inactive) or draining an entire bag (bubble sensor active: stop when dry). This is important to keep in mind when making/adjusting your protocol to ensure transfer of correct volumes and to ensure sufficient volume is in the reagent bags. See example below for two different ways of transferring the CTS™ Detachable Dynabeads™ Release Buffer. Also, see Chapter 3 (Embedded graphical user interface (eGUI) user instructions) in the *CTS™ DynaCellect™ Magnetic Separation System User Guide* (Pub. No. MAN0026480) for more details on how to make protocols.

Example: In a protocol where 9 mL of CTS™ Detachable Dynabeads™ CD4 and 4 mL of CTS™ Detachable Dynabeads™ CD8 is used, 65 mL CTS™ Detachable Dynabeads™ Release Buffer is required to release the CTS™ Detachable Dynabeads™ (5 mL CTS™ Detachable Dynabeads™ Release Buffer per mL of CTS™ Detachable Dynabeads™). The transfer step of 65 mL CTS™ Detachable Dynabeads™ Release Buffer to the bead-bound cells in the isolation bag can be programmed in several ways on the CTS™ DynaCellect™ Magnetic Separation System.

- **Transfer a set volume of CTS™ Detachable Dynabeads™ Release Buffer (bubble sensor inactive)** Program CTS™ DynaCellect™ Magnetic Separation System to pump 65 mL in addition to the dead volume within the CTS™ DynaCellect™ Cell Isolation Kit. As seen in Chapter 1 (Product information) in the *CTS™ DynaCellect™ Magnetic Separation System User Guide* (Pub. No. MAN0026480), the dead volume in the CTS™ DynaCellect™ Cell Isolation Kit from line F (where the CTS™ Detachable Dynabeads™ Release Buffer bag is located) to line A (where the bead-bound cells are located) is 16 mL. Thus, (65 + 16) mL = 81 mL should be transferred from F to A. As it is recommended to not introduce air into the lines of the CTS™ DynaCellect™ Cell Isolation Kit, 20% extra CTS™ Detachable Dynabeads™ Release Buffer volume should be in the bag. Therefore, the bag should contain at least 97 mL CTS™ Detachable Dynabeads™ Release Buffer.
- **Drain the CTS™ Detachable Dynabeads™ Release Buffer bag (bubble sensor active: stop when dry)** Program the CTS™ DynaCellect™ Magnetic Separation System to drain the CTS™ Detachable Dynabeads™ Release Buffer bag. The step where the CTS™ Detachable Dynabeads™ Release Buffer is transferred from line F to A should have the bubble sensor active to override the pump once it detects absence of liquid (Stop when dry). For this protocol, the exact volume of required CTS™ Detachable Dynabeads™ Release Buffer should be in the bag as the bag will be drained.

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. MAN1000204 A

Revision	Date	Description
A	29 August 2024	New document for CTS™ Detachable Dynabeads™ CD4, CTS™ Detachable Dynabeads™ CD8, CTS™ Detachable Dynabeads™ Release Buffer (Automated Workflow).