Cell therapy

Buffer exchange from CTS Detachable Dynabeads Release Buffer to CTS OpTmizer T-Cell Expansion SFM using the CTS Rotea system

Keywords
CTS DynaCellect Magnetic Separation System, CTS Detachable Dynabeads magnetic beads, CTS Rotea Counterflow Centrifugation System, buffer exchange, residual washing

Introduction

Chimeric antigen receptor T cell (CAR T) therapy has rapidly advanced from preclinical research to clinical applications for personalized medicine. However, wide application of this technology requires a streamlined manufacturing process to achieve reproducible high-quality, clinical-grade products.

The Gibco™ CTS™ DynaCellect™ Magnetic Separation System (Cat. No. A55867) is a flexible, automated, and closed system that enables rapid T cell isolation and magnetic bead removal for cell therapy manufacturing. Gibco™ CTS™ Detachable Dynabeads™ magnetic beads, such as CTS Detachable Dynabeads CD3/CD28 (Cat. No. A56996; for user guide, see [1]), are used for T cell isolation on the CTS DynaCellect Magnetic Separation System. The target T cells are released from the CTS Detachable Dynabeads magnetic beads using the CTS Detachable Dynabeads Release Buffer. For subsequent T cell expansion, the Detachable Dynabeads Release Buffer must be exchanged with Gibco™ CTS™ OpTmizer™ T-Cell Expansion SFM, and we recommend doing so using the Gibco™ CTS™ Rotea™ Counterflow Centrifugation System [2].

The CTS Rotea system is a highly versatile tool that can be utilized at multiple points during a cell therapy workflow, such as buffer exchange and residual washing. We have shown that the CTS Rotea system can effectively remove different residuals from the cell bed, providing consistent product purity to enable automation [3].

Here we describe a method for using the CTS Rotea system to exchange CTS Detachable Dynabeads Release Buffer with CTS OpTmizer T-Cell Expansion SFM.

Materials

- CTS Rotea Counterflow Centrifugation System (Cat. No. A47695)
- Gibco™ CTS™ Rotea™ Single-Use Kit (Cat. No. A49585)
- Terumo™ transfer bags
- CTS OpTmizer T-Cell Expansion SFM, no phenol red, bag format (Cat. No. A3705003; for media preparation, see user guide [4])
- Gibco™ CTS™ DPBS (Cat. No. A1285601) supplemented with 1% HSA
- Sterile tubing welder
- Tube sealer

Methods

1. Prepare the CTS Rotea Single-Use Kit.

   - Prepare input and output bags/vessels.
   - Use a sterile tubing welder to attach input and output bags/vessels to tubes A, B, C, D, G, and H of the CTS Rotea Single-Use Kit (Figure 1).

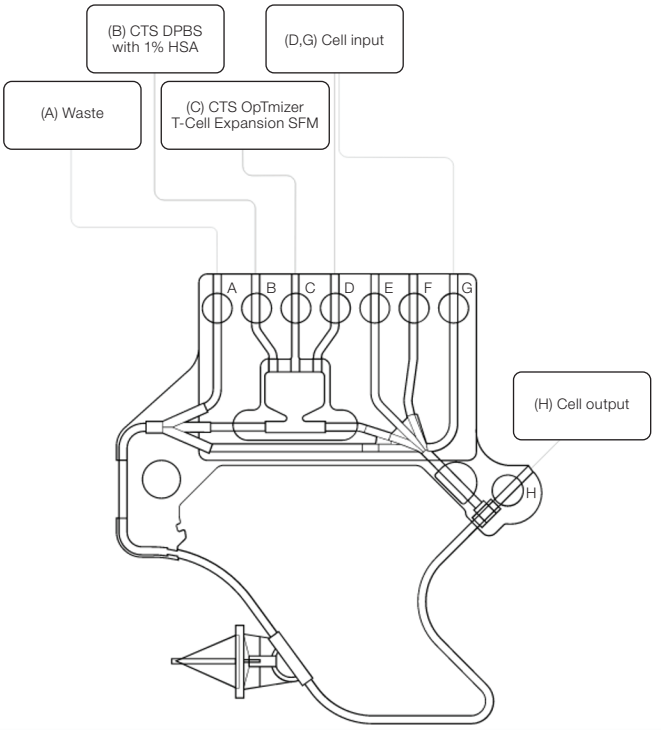

Figure 1. CTS Rotea Single-Use Kit configuration for buffer exchange.

2. Install the kit onto the CTS Rotea system.

   - Ensure that each tube is inserted into the corresponding slot in the Bubble Detector Strip, and push downward with your finger to fully engage each tube in the slot.
   - Stretch the pump tubing around the peristaltic pump roller and insert the tube retainer into the sensor block to hold it in place.
   - Minimum input (working volume) is ~50 mL.
   - Maximum bag weight per pole is ~4.4 lb (2 kg).

3. Initiate a protocol.

   - Open the CTS Rotea GUI software and select a protocol programmed as described in Table 1.
   - Confirm that the single-use kit's configuration matches the image in the dashboard of the CTS Rotea GUI software.
   - Ensure that all clamps have been released prior to starting the automatic sequence.
   - Enter correct data inputs:

     i. Draw volume: volume of cell suspension in input bag (D and G)

     ii. Harvest volume: depends on desired cell concentration in output volume

     iii. Wash volume: 150 mL wash buffer (DPBS with 1% HSA)

     iv. Establish bed volume: depends on input cell concentration; 10^8 cells are needed to establish the cell bed (e.g., if the input cell concentration is 1 x 10^6 cells/mL, the bed volume is 100 mL)

Table 1. Buffer exchange protocol.

Step	Description	Flow path	Centrifugal force (x *g*)	Flow rate (mL/min)	Step type	Trigger
1	Pre-prime	B to A	0	100	Normal	Input bubble sensor
2	Pre-prime chamber	B to A	0	100	Normal	10 mL
3	Fill chamber and prime A	B to A	10	100	Normal	45 mL
4	Fill bubble trap and prime B	A to B	10	100	Normal	15 mL
5	Prime D	A to D	10	100	Normal	5 mL
6	Prime C	A to C	10	100	Normal	5 mL
7	Pressure prime	A to E, F	10	0	Pressure prime	–
8	Prime recirculation	J to K	10	25	Pause	3 mL
9	Ramp pump	J to K	10	100	Pause	31 seconds
10	Ramp centrifuge	J to K	2,000	100	Pause	31 seconds
11	Pause before establish bed	J to K	2,000	40	Pause	31 seconds
12	Establish bed slow	D to G	2,000	20	Normal	Establish bed volume
13	Load cells	D to A	2,000	25	Normal	Draw volume, input bubble sensor, pause
14	Pause before wash	J to K	2,000	20	Pause	20 seconds
15	Wash	B to A	2,200	20	Normal	Wash volume
16	Concentrate bed	J to K	2,200	20	Pause	10 seconds
17	Harvest	C to H	2,200	50	Harvest	Harvest volume
18	Ramp to stop	K to J	500	50	Pause	31 seconds

4. Priming: protocol steps 1–10 (Table 1).
   - Press the Start button to start the protocol.
   - Visually inspect and ensure that a sufficient priming volume is pumped through the system.
   - Prime all tubes in which fluid will enter the system.
5. Cell loading: protocol steps 11–13 (Table 1).
   - Ensure that a stable fluidized bed has been established.
     i. The top surface of the bed is mostly flat with minimal turbulence.
     ii. Cells are not exiting the CFC chamber.
     iii. The stainless steel dip tube is visible at the bottom tip of the CFC chamber and between the top surface of the bed and the CFC chamber exit port.
     iv. Up to 5 x 10^9 T cells can be loaded in the CFC chamber using a high g-force and low flow rate. A total input of 2 x 10^9 to 3 x 10^9 cells is common.
     v. Cells less than 4 µm in size cannot be concentrated in the CFC chamber. As such, these cell types do not need to be counted when calculating the volume of input material.
     vi. When loading, allow enough space in the chamber to contain cells as the fluidized bed expands.
6. Wash and concentrate: protocol steps 14–16 (Table 1).
   - Wash: exchanges the release buffer with the wash buffer.
   - Concentrate bed: concentrates the fluidized bed to enable small-volume harvesting and provide an important step from which to restart the protocol, should there be an interruption during loading or harvesting.
7. Harvest: protocol steps 17–18 (Table 1).
   - Harvest step is enabled on line H.
   - Harvesting in CTS OpTmizer T-Cell Expansion SFM using the volume register, “Harvest Volume”, which needs to be selected prior to running the protocol.
8. Remove kit.
   - Seal all fluid lines using a tube sealer prior to unlocking the door.

Conclusion

Following this protocol on the CTS Rotea system, T cells are successfully transferred from the CTS Detachable Dynabeads Release Buffer to the CTS OpTmizer T-Cell Expansion SFM for downstream T cell expansion.

References

1. Thermo Fisher Scientific (2023) User guide: **CTS Detachable Dynabeads CD3/CD28 Kit**
2. Thermo Fisher Scientific (2022) User guide: **CTS Rotea Counterflow Centrifugation System**
3. Thermo Fisher Scientific (2022) Application note: **Residual washout on the CTS Rotea Counterflow Centrifugation System**
4. Thermo Fisher Scientific (2023) User guide: CTS OpTmizer T-Cell Expansion SFM

Learn more about the CTS Rotea system at thermofisher.com/rotea

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