Residual washout on the CTS Rotea Counterflow Centrifugation System

Introduction

Cells may be thawed, selected, expanded, genetically altered, differentiated, and/or cryopreserved prior to use for cell therapy. Each step may require a different medium or buffer that may contain substances that can harm the cells. The Gibco™ CTS™ Rotea™ Counterflow Centrifugation System can effectively remove residual substances. Wash buffer can be pumped through the fluidized bed to replace the medium used to load the cells, and over 95% of residual substances can be removed with minimal cell loss. Here we describe how the CTS Rotea system can be used to efficiently remove various residual substances from T cell culture medium. Washing T cells to remove impurities helps make the cells safer and more useful for downstream applications. The cell washing and concentration workflow for the CTS Rotea system is outlined below.

Workflow for the CTS Rotea Counterflow Centrifugation System.

T cell isolation and expansion	Washing and concentration	Analyze
• CTS Dynabeads CD3/CD28 beads • CTS OpTmizer T Cell Expansion SFM • CTS Immune Cell SR (serum replacement) • GlutaMAX Supplement • L-glutamine • IL-2 recombinant human protein	• CTS Rotea Counterflow Centrifugation System • CTS DPBS	• Cell counting • IL-2 and HSA measurement (ELISA) • DMSO measurement (GC) • Glucose measurement

Prime system	Load cells	Wash cells	Harvest

Materials and methods

Human T cells were initially isolated from peripheral blood mononuclear cells (PBMCs) stored in a leukopak using the CTS Rotea system and the Gibco™ CTS™ Rotea™ Single-Use Kit (Figure 1). After thawing, the T cells were isolated and expanded with Gibco™ CTS™ Dynabeads™ CD3/CD28 beads in T cell complete medium: Gibco™ CTS™ OpTmizer™ T Cell Expansion serum-free medium (SFM) supplemented with Gibco™ CTS™ Immune Cell serum replacement (SR), Gibco™ L-Glutamine, Gibco™ GlutaMAX™ Supplement, and interleukin 2 (IL-2). Following expansion, the T cells were washed and concentrated on the CTS Rotea system using Gibco™ CTS™ DPBS either alone or CTS DPBS supplemented with 2% human serum albumin (HSA) as a wash buffer. T cell recovery and viability were analyzed using a ChemoMetec™ NucleoCounter™ NC-200™ automated cell counter before and after processing. The parameters of the washing and concentration protocol for the CTS Rotea system are summarized in Table 1.

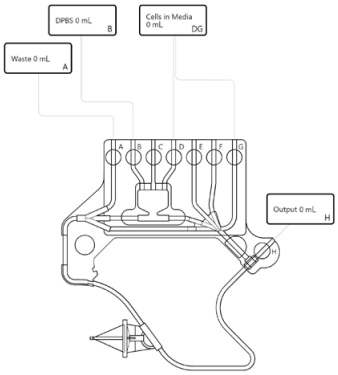


Figure 1. CTS Rotea Single-Use Kit configuration for washing and concentrating T cells.

Table 1. T cell washing protocol.

Step	Description	Flow path	*g*-force	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	40 mL
4	Fill bubble trap and prime B	A to B	10	100	Normal	15 mL
5	Prime D	A to D	10	30	Normal	5 mL
6	Pressure prime	B to E, F	10	0	Pressure prime	–
7	Prime recirculation	J to K	10	25	Pause	3 mL
8	Establish bed	D to G	2,000	20	Normal	80 mL
9	Load cells	D to A	2,000	25	Normal	Input bubble sensor
10	Wash	B to A	2,000	25	Normal	145 mL
11	Concentrate bed	J to K	2,200	20	Pause	10 sec
12	Harvest	B to H	2,200	50	Harvest	20 mL
13	Ramp to stop	K to J	500	50	Pause	5 sec

Table 2. Residual wash components, wash buffer compositions, and T cell inputs for washing and concentration on the CTS Rotea system.

Residual component	Concentration	Wash buffer	T cell input
Glucose	100–150 mM	DPBS + 2% HSA	2 x 10^8, 5 x 10^8, or 5 x 10^9 cells
IL-2	100 units/mL	DPBS + 2% HSA	1 x 10^9 cells
Human serum albumin	2%	DPBS	1 x 10^9 cells
DMSO	10%	DPBS	1 x 10^9 cells

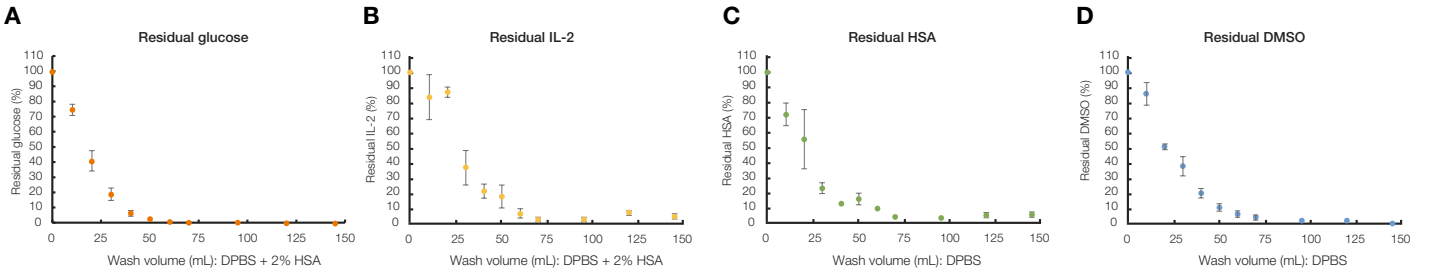
As the cells were being washed, the output was sampled through a tube leading to the waste bag using a syringe attached to a Y tubing connector (Figure 1). The residual glucose concentration was measured on a Nova BioProfile™ Flex2 cell culture analyzer. The residual IL-2 and human serum albumin (HSA) concentrations were determined using the Invitrogen™ IL-2 Human ELISA Kit, High Sensitivity (Cat. No. BMS221HS) and the Invitrogen™ Albumin Human ELISA Kit (Cat. No. EHALBX5). The concentration of residual DMSO in the output was measured via gas chromatography (GC). The residual components, concentrations of residual components, wash buffers, and initial T cell quantities are listed in Table 2.

T cells were also washed manually using a benchtop centrifuge. The T cells were washed with either 10 mL or 25 mL of wash buffer in a 50 mL conical tube by repeatedly centrifuging them at 300 x *g* for 5 minutes and resuspending them in wash buffer.

Results

Removal efficiency with varying volumes of wash buffer

More than 99% of residual glucose was removed from the medium by running 60 mL of DPBS + 2% HSA through the stable fluidized bed in the CTS Rotea chamber. No glucose was detected after washing the cells with 120 mL of the same wash buffer (Figure 2A). Over 95% of residual IL-2 was removed by washing the cells with 70 mL of DPBS + 2% HSA, and washing with 70 mL of DPBS without HSA removed over 95% of residual HSA and DMSO (Figures 2B–D).



Wash volume (mL)	Glucose removed
10	25.08%
20	58.96%
30	81.12%
40	93.81%
50	97.45%
60	99.19%
70	99.79%
95	99.98%
120	100.00%*

* No glucose detected in output.

Wash volume (mL)	IL-2 removed
10	16.72%
20	13.68%
30	63.54%
40	78.89%
50	82.61%
60	93.73%
70	97.25%
95	97.62%
120	93.11%
145	98.78%

Wash volume (mL)	HSA removed
10	28.01%
20	44.49%
30	76.65%
40	86.64%
50	83.89%
60	89.94%
70	95.62%
95	96.35%
120	94.11%
145	94.02%

Wash volume (mL)	DMSO removed
10	14.64%
20	49.19%
30	62.18%
40	80.12%
50	89.62%
60	93.85%
70	95.97%
95	97.91%
120	100.00%*

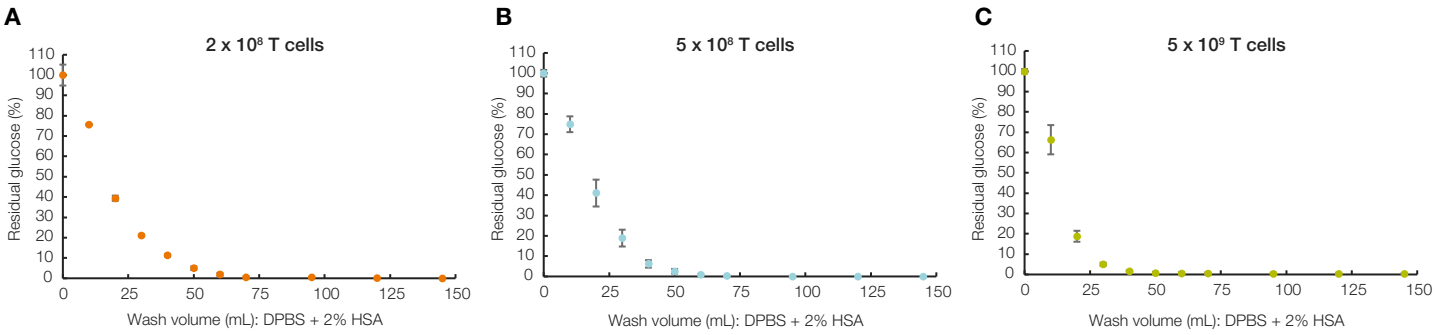
* Level comparable to negative control.

Figure 2. Washing efficiency of the CTS Rotea system. Percentage of **(A)** glucose, **(B)** IL-2, **(C)** HSA, or **(D)** DMSO removed from T cell culture medium.

T cell input

The efficiency of glucose removal was also evaluated for different quantities of T cells. The maximum processing capacity of the CTS Rotea system was 5 x 10^9 cells, and inputs of 2 x 10^8, 5 x 10^8, and 5 x 10^9 cells were tested.

Washing 2 x 10^8 or 5 x 10^8 cells with 70 mL of DPBS + 2% HSA removed 99% of residual glucose, and washing 5 x 10^9 cells with 50 mL of the same wash buffer removed >99% of residual glucose (Figure 3).



Wash volume (mL)	Removed glucose
10	24.31%
20	60.61%
30	78.89%
40	88.72%
50	95.05%
60	98.07%
70	99.57%
95	99.60%
120	99.89%
145	100.00%*

* No glucose detected in output.

Wash volume (mL)	Removed glucose
10	25.08%
20	58.96%
30	81.12%
40	93.81%
50	97.45%
60	99.19%
70	99.79%
95	99.98%
120	100.00%*

* No glucose detected in output.

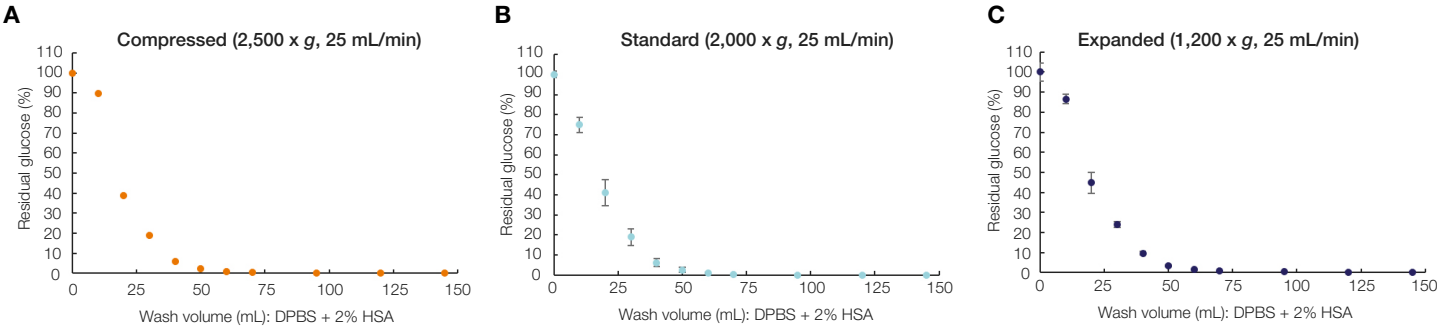
Wash volume (mL)	Removed glucose
10	33.68%
20	81.19%
30	94.94%
40	98.28%
50	99.18%
60	99.35%
70	99.49%
95	99.53%
120	99.58%
145	99.67%
245	99.99%

Figure 3. Glucose removal efficiency of the CTS Rotea system for varying T cell quantities. Percentage of glucose removed from medium containing **(A)** 2 x 10^8 T cells, **(B)** 5 x 10^8 T cells, or **(C)** 5 x 10^9 T cells.

Effect of the CF ratio

The impact of adjusting the CF ratio on glucose removal efficiency was also evaluated. The CF ratio was adjusted during Step 10 (Table 2) by setting the centripetal *g* force to 1,200 x *g*, 2,000 x *g*, or 2,500 x *g* at a constant flow rate of 25 mL/min.

The results were similar to those of the previous tests, and >99% of residual glucose was consistently removed by washing the cells with 70 mL of DPBS + 2% HSA (Figure 4).



Wash volume (mL)	Removed glucose
10	10.09%
20	61.14%
30	81.25%
40	94.15%
50	97.68%
60	99.20%
70	99.59%
95	100.00%*

* No glucose detected in output.

Wash volume (mL)	Removed glucose
10	25.08%
20	58.96%
30	81.12%
40	93.81%
50	97.45%
60	99.19%
70	99.79%
95	99.98%
120	100.00%*

* No glucose detected in output.

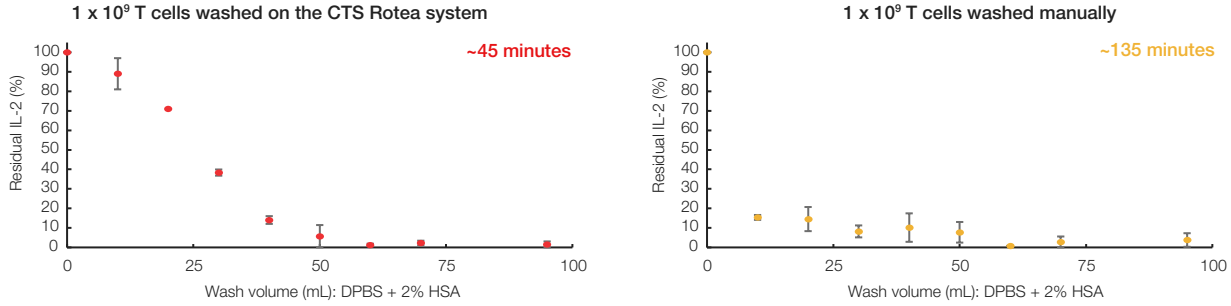
Wash volume (mL)	Removed glucose
10	13.48%
20	55.21%
30	76.09%
40	90.44%
50	96.74%
60	98.54%
70	99.28%
95	99.69%
120	99.77%
145	99.81%

Figure 4. The effect of CF ratio on glucose removal efficiency of the CTS Rotea system. Percentage of glucose removed at **(A)** 2,500 x *g*, **(B)** 2,000 x *g*, and **(C)** 1,200 x *g*.

Manual washing efficiency

We evaluated the residual IL-2 removal efficiency of the CTS Rotea system or manual washing, and the processing times of both methods were compared. The IL-2 removal efficiency of each method was similar; over 95% of residual IL-2 was removed by washing the cells with 70 mL of DPBS + 2% HSA.

While manual washing with 10 mL of the wash buffer removed ~85% of residual IL-2, 50 mL of wash buffer was needed to remove more than 90% of residual IL-2 (Figure 5). Manually washing cells with 145 mL of wash buffer took ~135 minutes, but washing on the CTS Rotea system was completed in ~45 minutes.



Wash volume (mL)	IL-2 removal on the CTS Rotea system	IL-2 removal by manual washing
10	11.32%	84.62%
20	29.76%	85.47%
30	61.64%	91.79%
40	85.95%	89.83%
50	94.27%	92.25%
60	98.74%	99.23%
70	97.74%	97.19%
95	98.36%	96.20%

Figure 5. Comparison of the IL-2 removal efficiency of the CTS Rotea system and manual washing with a benchtop centrifuge.

Cell recovery and viability

Excellent cell viability and high recoveries of processed T cells were observed under all test conditions. Cell viability and recovery were consistently above 90% (Figure 6). For ways to improve cell recovery, see the troubleshooting guide on page 6.

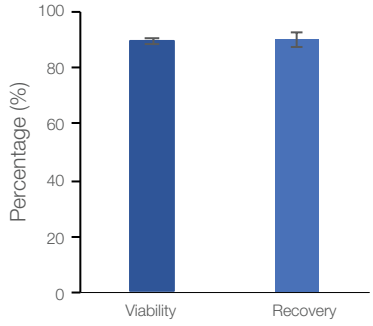


Figure 6. Average T cell viability and recovery after the washing and concentration process.

Medium exchange

The washing efficiency of the CTS Rotea system was also visualized while exchanging the medium. Green dye was added to T cell complete medium prior to the washing process. The green color gradually faded as more DPBS was delivered to the CTS Rotea chamber, and the dye was nearly completely removed by washing with 60–70 mL of the wash buffer (Figure 7).

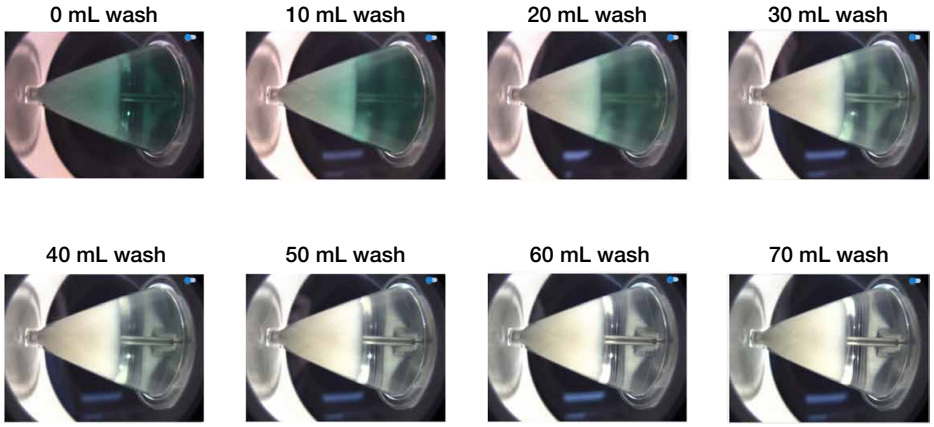


Figure 7. Visualization of medium exchange on the CTS Rotea system.

Conclusion

The CTS Rotea system can effectively remove residual substances from cell culture medium with minimal reduction in cell recovery and viability. We found that washing with 70 mL of wash buffer could remove >95% of common residual substances like DMSO and glucose. Comparable washing efficiency was observed when testing different residual components, cell inputs, and CF ratios. The CTS Rotea system can remove over 95% of residual substances three times as fast as manual washing. The wash steps performed in this study can be incorporated into any CTS Rotea workflow to remove impurities from T cells to facilitate downstream applications.

Troubleshooting guide

Step	Description	Possible cause	Adjustment
10	The fluidized bed is unstable or cell recovery is low.	Difference between the densities of the original medium and wash buffer.	If the wash buffer is dense or highly viscous, increase the *g*-force and/or reduce the flow rate. If the medium has a low density, increase the *g*-force and/or reduce the flow rate.
			If the fluidized bed becomes unstable, pause the protocol to allow it to settle.
			Add an intermediate bag to pre-dilute the cells with fresh wash buffer before washing. Pump fresh wash buffer into the intermediate bag for pre-dilution. After loading the cells, recover them as a concentrate in the intermediate bag. Re-establish the fluidized bed by recirculating and reload the diluted cells in the intermediate bag. Continue washing with fresh medium.
			Wash the cells while directing the output to an input bag. Once the cells are loaded, wash them. During the wash step, collect the output in a dual-port input bag instead of directing it to a waste bag. Re-establish the fluidized bed by recirculating and reload the diluted cells in the input bag. Continue washing with fresh medium.

Ordering information

Product	Quantity	Cat. No.
T cell culture		
CTS OpTmizer T Cell Expansion SFM, bottle format	1,000 mL	A1048501
CTS OpTmizer T Cell Expansion Supplement	26 mL	A3747001
CTS Immune Cell SR	50 mL	A2596101
GlutaMAX Supplement	100 mL	35050061
L-Glutamine (200 mM)	100 mL	25030081
Human IL-2 Recombinant Protein	1 mg	PHC0023
CTS DPBS (1X)	1 L	A1285801
CTS Dynabeads CD3/CD28	10 mL	40203D
Analysis		
IL-2 Human ELISA Kit, High Sensitivity	96 tests	BMS221HS
Albumin Human ELISA Kit	96 tests	EHALB
Cell washing and concentration		
CTS Rotea Counterflow Centrifugation System + 2 year warranty (including PM)	1 each	A50757† A47695‡
CTS Rotea Single-Use Kit	10 pk	A49585

† North America and Europe.

‡ Rest of world.

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