

## General purpose centrifuges

# Downstream processing of primary T cells using Thermo Scientific General Purpose X Series Centrifuges

## Keywords

T cell, T cell concentration, downstream processing, general purpose centrifuge, gp centrifuge, X series, X centrifuge

## Introduction

Washing and concentrating cells is one of the main steps in cell therapy research and manufacturing. During this step, expanded cell cultures are concentrated and washed in suitable buffer solutions to prepare for downstream steps, such as fill and formulation. Batch centrifugation has been the primary method for many years now. There have also been alternative methods emerging over the years, such as continuous-flow centrifugation, acoustic-based separation, and tangential flow filtration.

Here we show advantages of batch centrifugation and innovative solutions using the Thermo Scientific™ General Purpose X Series Centrifuges that can be optimized for downstream processing of primary T cells, and share some exemplary results for viability and recovery of T cells after washing and concentration.

## Fit-for-purpose equipment features for cell therapy manufacturing applications

### Secure and total control: SECURE-Spin mode

Thermo Scientific™ SECURE-Spin™ mode offers an advanced user management system on the centrifuge (Figure 1). Users are assigned different roles and privileges to maintain secure control, a feature which supports FDA 21 CFR Part 11 compliance.

The advanced functions of the SECURE-Spin mode allow you to:

- Register up to 120 individuals with unique user names and passwords
- Assign individual privileges and roles to each user, with different authorities
- Track all user changes to programs or settings with an audit trail

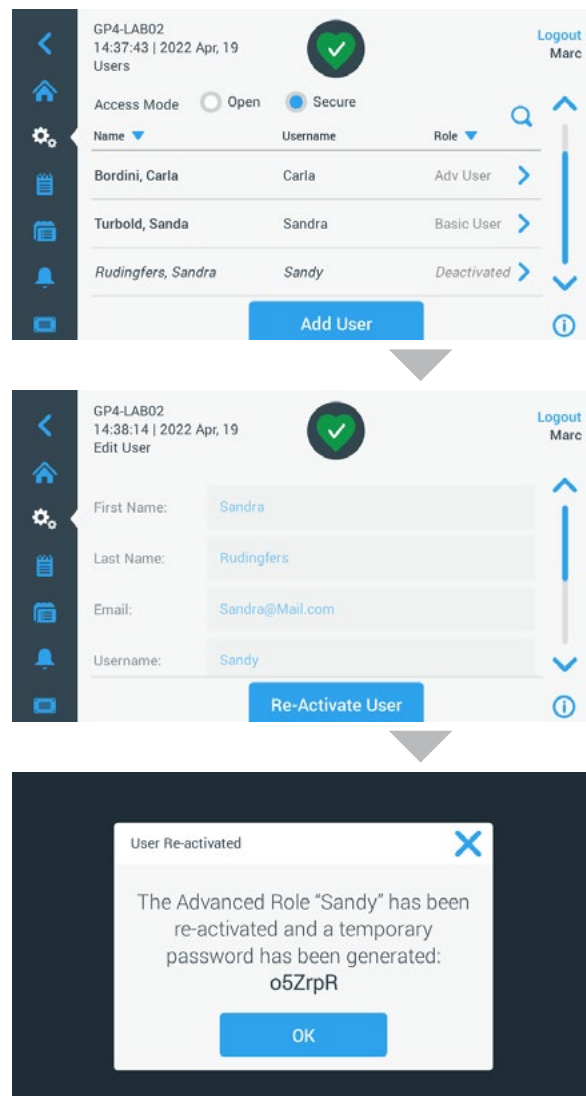


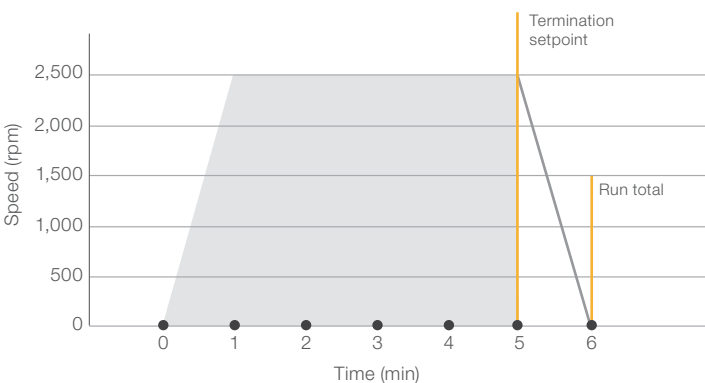
Figure 1. An example of the advanced user management system—steps for “re-activating” an advanced user profile.

## Reproducibility: ACE integrator function

The Thermo Scientific™ Accumulated Centrifugal Effect (ACE™) integrator function has been engineered to help ensure different batches of samples receive the same desired centrifugal effect, regardless of the weight of sample load, equipment age, or environmental factors, such as altitude (Figure 3). The ACE function is a mathematical model that helps to transfer applications and parameter settings between centrifuges by automatically modifying the run duration, as a substitute of time (Figure 2). This function can be enabled by navigating to “Settings”, then “Controls”, and then changing the setpoint mode to “Advanced”.

ACE function activation for the General Purpose X Series Centrifuges:

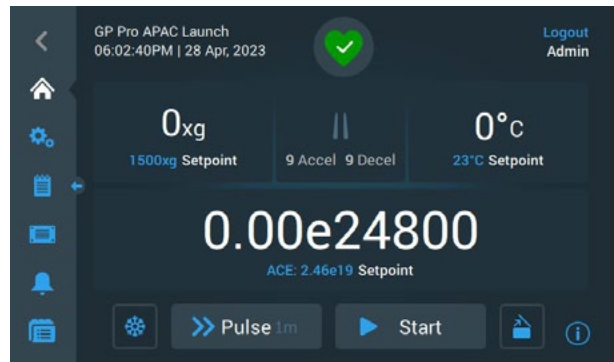
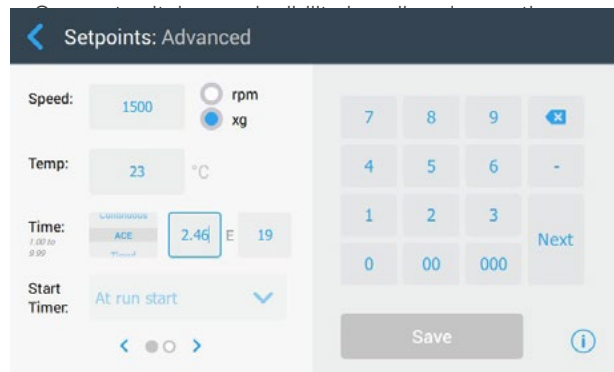
- Helps enable the same total accumulated centrifugal force regardless of conditions (e.g., time of run, rotor loading, sample load)
- Gives reproducible yields by automatically compensating for variations in acceleration by adjusting the centrifuge run time



**Figure 2. Benefits of the ACE integrator function.** The ACE function helps ensure that all samples experience the same total centrifugal effect (the area under the speed/time curve) by adding or subtracting time to a run.

### How to determine the ACE value

1. To start with, a working protocol is needed in which speed and run time are known.
2. In the ACE mode, enter the optimal speed value and a “high” ACE value. The higher value is to ensure that the run is not prematurely stopped.
3. Start a stopwatch at the start of the centrifugation run.
4. Stop the centrifuge when the time on the stopwatch has reached the known run time.
5. Record the ACE value shown on the screen.



**Figure 3. Setting the ACE integrator function value.**

## Contamination prevention

The Thermo Scientific™ ClickSeal™ Biocontainment Lids protect the samples from contaminating each other, enabling centrifugation of various samples in the same run. In case of any spillage events, the sample is contained within the buckets, which protects samples in other buckets, as well as users and the laboratory environment.

Key features of ClickSeal Biocontainment Lids:

- Ergonomically designed for easy opening and closing using only one hand, with either left or right hand
- Biocontainment sealing options for glove-friendly use
- Simple operation for all laboratory users, with the elimination of multi-turn screw caps and complicated high-pressure clips
- Biocontainment certification by the National Institute for Health Protection, Porton Down, UK



Thermo Scientific™ TX-1000 Swinging Bucket Rotor with ClickSeal Biocontainment Lids

## Health status

In cell therapy research and manufacturing of cells, particularly the autologous type, where cells are not readily available, promptness is a key for success. Thus, laboratory equipment must be ready to function flawlessly when needed. The “Health status” button on our centrifuges tracks and records each alarm and alert notification and rotor life cycle (Figure 4). This feature prevents downtime, which is vital for process continuity.

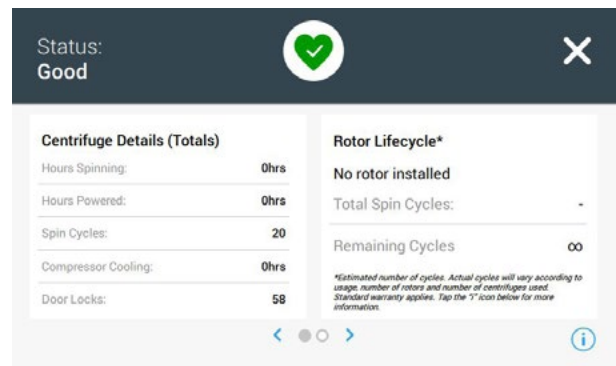


Figure 4. Centrifuge and rotor health in detail in health status screen.

### Additional benefits with Thermo Scientific™ SMARTSpin™ Technology

Advanced rotor management system maximizes acceleration, deceleration and residual load imbalance for each rotor and bucket option. X Series Centrifuges further enable fine-tuning of centrifugation parameters with high precision—increments of 1 rpm for speed and 1s for time. Furthermore, gentle acceleration and deceleration behaviors help maximize pellet integrity while keeping up process efficiency. In the example below, we employed acceleration profile 9 and deceleration profile 7 to minimize cell loss and maintain viability.

### Viability and recovery of T cells

#### Methods

Primary T cells provided by healthy donors were transferred into 50 mL and 500 mL conical containers at a concentration of  $1 \times 10^6$  cells/mL. Cell counting was performed using the Via2-Cassette™ device and NucleoCounter™ NC-200™ instrument (ChemoMetec; Allerød, Denmark). Containers were centrifuged in the Thermo Scientific™ X4TR general-purpose centrifuge at  $400 \times g$  at room temperature for 10 minutes. Viability and recovery of T cells were determined after washing and concentration.

Note: The cell concentration of  $1 \times 10^6$  cells/mL used is 4- to 5-fold lower than usual values, to simulate a worst-case scenario.

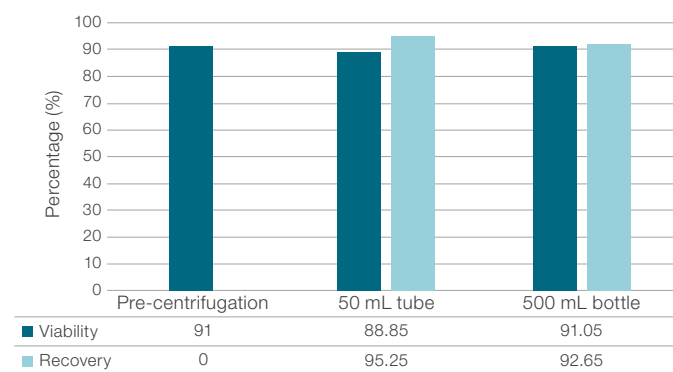


Figure 5. Viability and recovery of T cells.

### Results

Our results suggested that both conical container types, 50 mL and 500 mL, performed equivalently in concentrating T cells, with a minimal decrease in viability and high recovery rates (Figure 5).

### Conclusions

Batch centrifugation has been the workhorse of cell therapy manufacturing for decades. Based on our findings, we conclude it is the preferred method because of the following advantages:

- No limitation on applicable cell types
- No clogging when high cell densities are processed
- High performance with low cell density
- Active control and maintenance of process temperature
- Low capital investment, low cost of maintenance and operation
- Uncomplicated process development
- Flexibility to be used for numerous laboratory applications



Thermo Scientific X4FR and X4TR General Purpose Centrifuges

Learn more at [thermofisher.com/centrifuges](https://thermofisher.com/centrifuges)

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