

Effective applicability of Thermo Fisher Scientific General Purpose Centrifuges with TX-1000 - maximum capacity swinging buckets rotor in cell culture application

Authors: David Klinkenberg, PhD
Cell Culture Scientist & Technical Advisor, Annette R. Madsen, Lab. Tech., Tina K. Marwood, PhD Research Manager, Yuliya Mikhed, PhD, Applications Specialist, Centrifugation

Introduction

Cell culture is one of the major tools used in cellular and molecular biology, providing an excellent model for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), the effects of drugs and toxic compounds on the cells, mutagenesis and carcinogenesis. It is also used in drug screening and development, and large scale manufacturing of biological compounds (e.g., vaccines, therapeutic proteins). Additionally, cell culture is an invaluable tool for studying pathological conditions of the cell that can later on be translated to the tissue and organismal level.

Starting from the entry of the frozen cell vial of the cell culture into the experimental setting and continuing with the cell culture propagation and expansion - general purpose centrifuges and various rotors and adaptors are enabling researchers to achieve desirable results.

Safety, productivity, intuitive operation and versatility are key features that stand out for customers using Thermo Scientific general purpose centrifuges, in particularly using our currently largest capacity swinging buckets rotor - TX-1000.

High variability in the cell lines' sensitivity towards centrifugal forces requires that researchers determine centrifugation conditions cell line by cell line.

This application brief describes a procedure where Thermo Scientific™ General Purpose Centrifuge and TX-1000 swinging buckets rotor and 75007306 and 75003674 adapters examine the sensitivity towards centrifugation of CHO-K1 cell line in suspension cultures.



TX-1000 Swinging Bucket Rotor

Procedure

CHO suspension cell culture is now more common as a tool for producing and examining various biopharmaceutical peptides. In order to help researchers introduce this versatile tool and possibly ease the transition to this technique, we highlight here a protocol for examining CHO-K1 cells' sensitivity towards centrifugation using Thermo Scientific general purpose centrifuge with the TX-1000 swinging buckets rotor. The study examined the effect of different centrifugation forces (800 x g, 600 x g, and 200 x g) on pelleting efficiency, as well as cell viability as a key parameters.

The study extended an internal benchmarking study of Fisherbrand™ and Thermo Scientific™ Nalgene™ Shaker Flasks when compared to Manufacturer C Shaker Flasks, using Manufacturer C's product performance as a base line:

The procedure for cell propagation and expansion was performed as follows:

1. CHO-K1 cells were thawed and counted. Additionally, cell viability of the sample was determined. Viability was found to be 99.0% and considered to be acceptable to proceed further.
2. Cells were transferred into 125 mL manufacturer C shaker bottles and filled up to 25 mL with Thermo Scientific™ FortiCHO™ Complete medium and afterwards placed on a shaker at 125 rpm inside an incubator and maintained at 10% CO₂ and 37°C.
3. Cells were passaged 3 times in manufacturer C's flask.
4. After the third passage cells were split into three flasks each of the following brands – Fisherbrand™, Thermo Scientific™ Nalgene™ and Manufacturer C brand.
5. The cells from individual flasks were counted after 3 & 6 days. Performance was determined by comparing cell counts and viability between the three brands of flasks.
6. The content of all three flasks from a single brand of a flask was pooled into one flask of the same make, and a new count of cell concentration and viability was performed for each population to provide a common base line for the cell populations and to counteract cell count differences arising from the previously performed tests.

7. 2 x 3 mL aliquots of each cell population were transferred to 15 mL Thermo Scientific™ Nunc™ Centrifuge Tubes, and 3 x 20 mL aliquots were transferred to 50 mL Nunc™ Snap Cap Conical Tubes.
8. The 15 mL tubes were centrifuged at either 800 or 600 x g for 5 min at 8°C, and the pellet was re-suspended in 2 mL DPBS (pH=7.2).
9. The 50 mL tubes were centrifuged at either 800, 600 or 200 x g for 5 min at 8°C and the pellet was re-suspended in 5 mL DPBS (pH=7.2).
10. An aliquot of each suspension was removed for cell counting.
11. The total number of cells and viability percent was determined using a NucleoCounter™ NC-100 Cell Counter together with associated cassettes, lysis and neutralization buffers.



15 mL conical tube with pellet sedimentation



50 mL conical tube with pellet sedimentation

Cell Viability Results

	Brand	Centrifuge Tube Vol. (mL)	Vol. Centrifuged (mL)	Vol Resuspended (mL)	Living (u/mL)	Viability
Baseline	Manufacturer C				1.19E+08	99.99%
	Fisher Scientific				1.37E+08	99.98%
	Nalgene				1.03E+08	99.82%
800 x g	Manufacturer C	15	3	2	1.82E+08	99.70%
		50	20	5	4.80E+08	99.71%
	Fisher Scientific	15	3	2	1.49E+08	99.65%
		50	20	5	4.40E+08	99.81%
	Nalgene	15	3	2	1.45E+08	99.13%
		50	20	5	3.99E+08	99.62%
600 x g	Manufacturer C	15	3	2	1.44E+08	99.58%
		50	20	5	4.53E+08	99.77%
	Fisher Scientific	15	3	2	1.49E+08	99.72%
		50	20	5	3.81E+08	99.81%
	Nalgene	15	3	2	1.22E+08	99.29%
		50	20	5	3.48E+08	99.49%
200 x g	Manufacturer C	50	20	5	4.32E+08	99.90%
	Fisher Scientific	50	20	5	3.42E+08	99.79%
	Nalgene	50	20	5	3.66E+08	99.65%

Results

Visual observations and cell viability measurements on NucleoCounter™ NC-100 cell counter imply that the CHO-K1 cells do tolerate centrifuge forces up to 800 x g, showing viability above 99%. The data also implies that there is a minimal if any loss of cell material when spinning samples at 200 x g as opposed to 800 x g.

Choice of the tube volume – 15 or 50 mL will mostly depend on the size of the flask which researchers would like to split, making 15 mL tube useful for the smaller vessel volumes and 50 mL conical tube useful for bigger vessels.

Differences have been previously observed in terms of cell pelleting quality with other cell types than CHO-K1 using other centrifugation forces than those tested in our settings. Higher RCF values did cause cell rupture and should therefore be evaluated if absolutely necessary or preferably omitted. Our preference for the centrifugation conditions would therefore be 200 x g for 5 min at 8 °C.

Conclusions

Centrifugation with Thermo Scientific general purpose centrifuge and our currently largest capacity TX-1000 swinging buckets rotor gives appropriate tube coverage. Various adaptors for this rotor enable simultaneous spinning of 40 x 50 mL conical tubes and/or up to 96 x 15 mL conical tubes. Additionally, one may mix and match the adaptors, in order to tailor them to the specific customer needs. We achieved satisfactory CHO-K1 cell pelleting with 200 x g after 5 min of centrifugation at 8°C and recommend these conditions for further use by our customers.

Find out more at [thermofisher.com/centrifuges](https://www.thermofisher.com/centrifuges)

ThermoFisher
SCIENTIFIC

This product is intended for General Laboratory Use. It is the customer's responsibility to ensure that the performance of the product is suitable for the specific use or application. © 2021 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. NucleoCounter is a trademark of ChemoMetric A/S.

COL34558 1121