Cell Separation

Beckman Coulter Cell Culture Flask Adapters.

Introduction

During various stages of the cell culturing process, centrifugation is frequently used to isolate extracellular products or to separate cells from their aqueous environment. Traditionally, cells and media are first transferred from a cell culture flask to a 15 mL or 50 mL conical tube before centrifugation. These transfer steps require operator labor and time, and the cost of the transfer vessel, and also introduce the potential for contamination during transfer. With the innovative Cell Culture Flask Adapters,* the culture can be centrifuged directly in the flask. Data illustrate that cell yield, cell viability, and endpoint analysis results are comparable when cell cultures are processed traditionally or centrifuged directly in the flask using Cell Culture Flask Adapters.

Major Benefits of the Cell Culture Flask Adapters

— Streamlined cell culturing process.

— Time, labor, and labware savings.

— Reduced potential for contamination.

What are Cell Culture Flask Adapters?

The Cell Culture Flask Adapters are single-piece elastomeric (EPDM) adapters designed to allow centrifugation of T-75† (75 cm^2 area) or T-25† (25 cm^2 area) cell culture flasks in the Allegra X-14R, Allegra X-15R and Allegra X-12 Series centrifuges using SX4750 and SX4750A rotors (Fig. 1). The adapters stand up to the rigors of use (centrifugal force, cleaning, and sterilization) in the bioresearch laboratory.

Figure 1. Beckman Coulter Cell Culture Flask Adapters.

When to Use the Cell Culture Flask Adapters

Cell Culture Flask Adapters support cell culture flasks during centrifugation. Centrifugation is used in the cell culturing process (seeding, passaging, or harvesting) and in studies of cellular activities (organelles, proteins, antibody production, etc.) and cellular products. During various phases (cell cycle, adhesion, motility, signal transduction, etc.) of the cellular study, centrifugation is used to separate or concentrate cells from culture media and/or to collect extracellular products.

Traditionally, the cells and media are transferred from a cell culture flask to a container (typically a 15 mL or 50 mL conical tube). After centrifugation, a cell pellet is either resuspended and transferred back to the cell culture flask for continued growth, or it is extracted for storage or downstream analysis. These transfer steps can be eliminated when culturing and centrifugation are carried out in the same flask using the Cell Culture Flask Adapters. The streamlined process is applicable for various types of cell cultures (adherent, suspension, hybridoma, and primary cells; see Fig. 2).

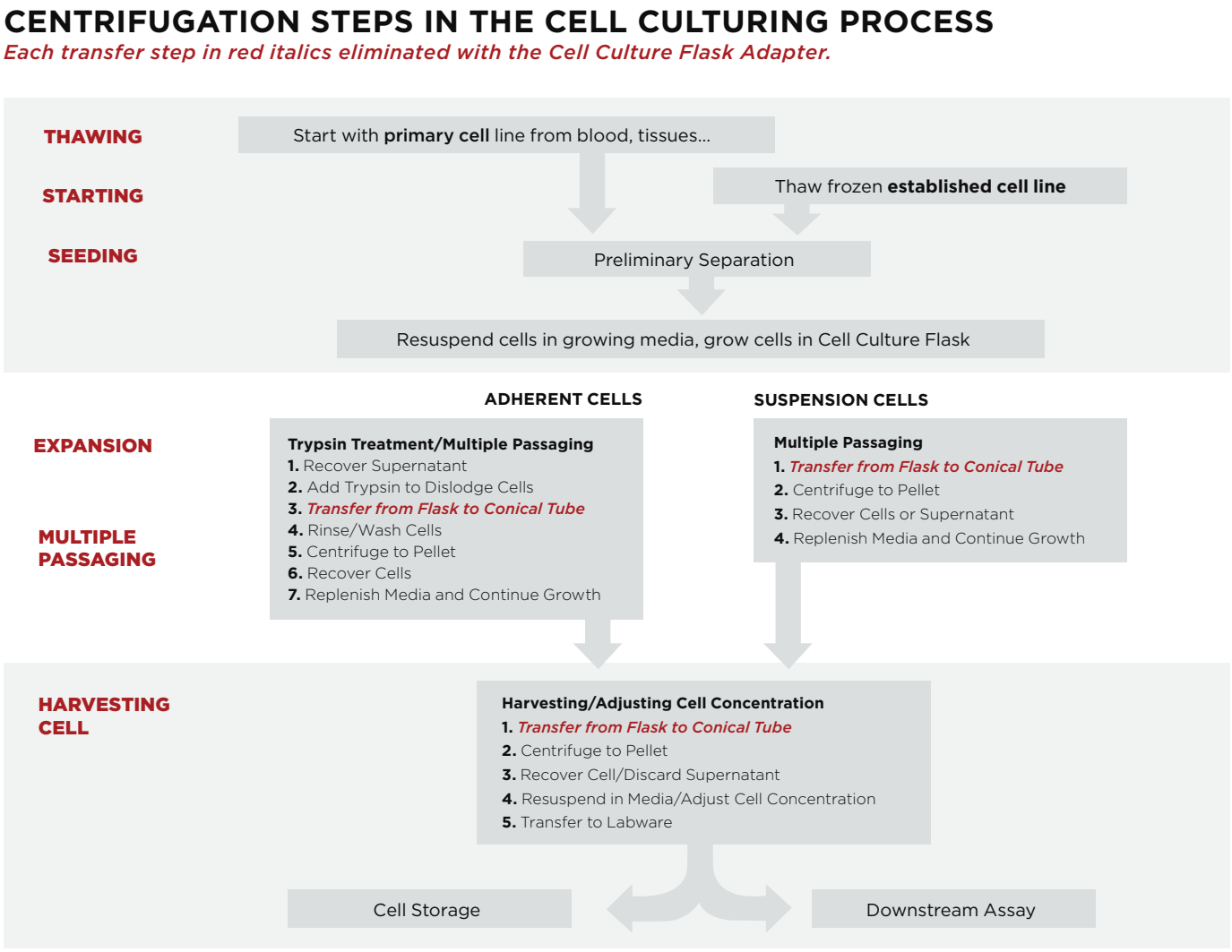


Figure 2. Centrifugation steps in the cell culturing process.

Comparable Results

Comparable results (Tables 1 and 2) have been obtained when cell cultures are processed traditionally or centrifuged directly in flasks using the Cell Culture Flask Adapters.

— Comparable cell viability.

— Comparable endpoint analysis results.
  – Viral PFU counts
  – Monoclonal antibody production
  – Cytokine protein measured by ELISA
  – Fluorescence detection of caspase from an apoptosis study

— Comparable cell yield—for optimization, Cell Culture Flask Adapters can be centrifuged at a speed greater than that typically run in a 15 mL or 50 mL conical tube for cell concentration. This process can be performed without disruption of cells.

Table 1. Comparison of Endpoint Analysis Results

CONICAL TUBE VS. CELL CULTURE FLASK CENTRIFUGATION				
Cell Line	**Culture Type**	**Endpoint Analysis**	**Results**	
			Tube	**Cell Culture Flask (T-75)[11]**
Vero	Adherent	Viral Titer HSVI production from culture media of infected vero cells.	4.5E8 PFU[1]	1.8E8 PFU
Hybridoma 2E7 line 5E5 line	Suspension	Hybridoma cell line for monoclonal antibody production. The SUP,[2] after filtering through a 0.2 µm filter, was applied to a column.[4] The OD recovery was measured on the effluent as reference to MAB[5] production/ purification.	4.5 OD[3] / 5 mL SUP[2] 0.9 mg MAB / 450 mL SUP 2.1 mg MAB / 450 mL SUP	4.5 OD / 5 mL SUP 0.7 mg MAB / 450 mL SUP 1.7 mg MAB / 450 mL SUP
Umbilical Cord Blood Primary Culture	Suspension Interleukin – ß (NT[6]) Interleukin – ß (PHA[7]) Interferon - γ (NT) Interferon - γ (PHA) Interleukin - 4 (NT) Interleukin - 4 (PHA)	Cord Blood → Ficoll Separation, Cytokines from cell culture as measured by ELISA.	(pg / mL antibody) 747 4,472 1 684 1 10	 722 3,597 17 624 1 25
HeLa	Adherent Caspase 3 (NT) Caspase 3 (STS)	Drug (STS[8]) induced apoptosis causes cell (4 x 10^3 cells/microplate well) to accumulate enzyme, measured by fluorescence assay.	123 FIU[9] ± (50)[10] 91,000 FIU ± (1200)	76 FIU ± (25) 108,000 FIU ± (1430)

1. PFU: Plaque Forming Units.
2. SUP: Supernatant after centrifugation.
3. OD: Optical density in spectrophotometric absorbance units at 280 nm.
4. Column: Thiophilic Super Flow resin column from Clontech.
5. MAB: Monoclonal Antibody.
6. NT: No Treatment as Control.
7. PHA: Phytohemagglutinin, a T-cell mitogen.
8. STS: Staurosporin (a protein Kinase C inhibitor), 1 µg/mL treated for 6 hrs.
9. FIU: Fluorescence Intensity Units.
10. ± ( ): ± standard deviation.
11. T-75: Corning 75 cm^2 canted-neck cell culture flasks.

Table 2. Cell Yield and Viability of Different Cell Types and Culturing Stages

COMPARISON OF CONICAL TUBE AND CELL CULTURE FLASK CENTRIFUGATION							
Cell Type	**Cell Culture Flask[1]**	**Centrifugation Parameters[2]— RPM (x *g*) / Time (min)**	**Culture Stages[3]**	**Cell Yield**		**Cell Viability**	
				Tube	**Cell Culture Flask**	**Tube**	**Cell Culture Flask**
HeLa Adherent 10 mL culture	T-75	1200 (329) / 10	S	1.6E6 / 5 mL	1.2E6 / 5 mL	87%	78%
			P	9.8E6 / 5 mL	1.0E6 / 5 mL	99%	100%
			H	2.1E6 / 5 mL	2.0E6 / 5 mL	95%	95%
		1200 (335) / 10	H	1.2E8 / 5 mL	1.0E8 / 5 mL	ND[4]	ND
			H	1.0E8 / 5mL	9.8E7 / 5 mL	ND	ND
	T-25	1200 (329) / 10	P	2.1E6 / mL	2.3E6 / mL	98%	99%
		1200 (335) / 10	H	2.9E7 / mL	2.0E7 / mL	ND	ND
			H	2.6E7 / mL	2.0E7 / mL	ND	ND
Jurkat Suspension 10 mL culture	T-75	1200 (329) / 10	S	2.5E6 / 5 mL	1.8E6 / 5 mL	30%	20%
			P	6.0E6 / 5 mL	5.3E6 / 5 mL	96%	94%
			P	3.1E6 / 5 mL	2.9E6 / 5 mL	96%	92%
		1700 (660) / 15	P	10.6E6 / 5 mL	8.3E6 / 5 mL	91%	88%
		1200 (335) / 10	H	3.0E7 / 5 mL	3.1E7 / 5 mL	ND	ND
			H	3.0E7 / 5 mL	3.1E7 / 5 mL	ND	ND
	T-25	1200 (329) / 10	P	2.1E6 / mL	1.5E6 / mL	98%	98%
		1200 (335) / 10	H	7.6E6 / mL	7.7E6 / mL	ND	ND
			H	7.5E6 / mL	6.7E6 / mL	ND	ND
Mononuclear Cell Prep from umbilical cord blood	T-75						
	No Treatment	1200–1500 (266–416) / 10	P	84% PT[6]	ND	97%	ND
		1200–1500 (329–514) / 10	P	ND	92% PT[6]	ND	98%
	PHA[5] Stimulated	1200–1500 (266–416) / 10	P	80% PT[6]	ND	98%	ND
		1200–1500 (329–514) / 10	P	ND	88% PT[6]	ND	95%
Monocyte (mononuclear cell) from umbilical cord blood, adherent, 10 mL culture	T-75	1500 (416) / 5	P	96% PT[7]	ND	ND	ND
			H	97% PT[7]	ND	ND	ND
		1500 (514) / 5	P	ND	99% PT[7]	ND	ND
			H	ND	93% PT[7]	ND	ND
Lymphocyte (mononuclear cell) from umbilical cord blood, 16 mL suspension	T-75						
	No Treatment	1200–1500 (266–416) / 10	P	16% PT[6]	ND	ND	ND
		1200–1500 (329–514) / 10	P	ND	8% PT[6]	ND	ND
	PHA Stimulated	1200–1500 (266–416) / 10	P	20% PT[6]	ND	ND	ND
		1200–1500 (329–514) / 10	P	ND	12% PT[6]	ND	ND

1. T-75: Corning 75 cm^2 canted-neck cell culture flasks; T-25: Corning 25 cm^2 canted-neck cell culture flasks were used for the experiments.
2. All centrifugation runs conducted in room temperature (18º C–25º C). Centrifugation runs with the conical tubes and cell culture flasks were conducted at the same speed and for the same duration of time.
3. Stages: S (Release from Frozen **S**torage); P (**P**assaging, Reseeding, Expansion); H (**H**arvesting).
4. ND: No Data.
5. PHA: Phytohemagglutinin, a mitogen for T-cells.
6. % cell PT = 100% - [(cell in supernatant) / (total cell) x 100].
7. % cell PT = (cell in pellet) / (total cell) x 100.

How to Use the Cell Culture Flask Adapters

Filled flasks can easily be inserted into and extracted from the Cell Culture Flask Adapters (Fig. 3).

Before first use and after cleaning the adapter, lightly coat the inside of the bucket with dry release agent (P/N 392819) to facilitate the removal of the adapter from the SX4750 or SX4750A round bucket.

The orientation of the flask and adapter with respect to the rotor is shown in Fig. 4. Extraction of the flask from the adapter is easy with minimal disturbance of the pellet.

Figure 3. Flasks are easily inserted into the adapter. With adapters for the Corning 75 cm² canted-neck flasks, insert with thick-seam side facing the direction of the arrows on the adapter top; or insert the canted-top tilted toward the side of the adapter with the arrows. Adapters for Corning 25 cm² canted-neck flasks can tilt in either direction.

Figure 4. Easily load the adapter into the bucket by grasping the adapter finger grips. Align the narrow sides of the flask to the bucket engraving and the wide sides parallel to the bucket pin pockets.

Expect a Faster Spin

Higher centrifugal force (up to 3,000 rpm/2,000 x *g*) may be used when centrifuging flasks in the Cell Culture Flask Adapters to optimize cell yield (Table 3).

Various types of cell cultures (adherent, suspension, and primary tissue) have been shown to maintain good viability (> 90%) and cellular function when spun at higher forces versus traditional processing (Table 2).

Table 3. Effect of Centrifugal Speed (RPM) on Cell Yield and Viability

EFFECT OF CENTRIFUGAL SPEED (RPM) ON CELL YIELD AND VIABILITY					
Cell Type	Centrifugation Speed[1] (rpm) 10 Minutes	Cell Yield[2]		Cell Viability	
		Tube	Cell Culture Flask[3]	Tube	Cell Culture Flask[3]
ATCC K562	1200	104%	48%	97%	97%
Suspension	2750	117%	114%	95%	92%
Prostate Cell Line	1200	180%	64%	93%	92%
Adherent	2500	133%	115%	92%	92%
Vero Cell	1100	85%	84%	Comparable	
Adherent, 25 mL	2500	148%	107%	Comparable	

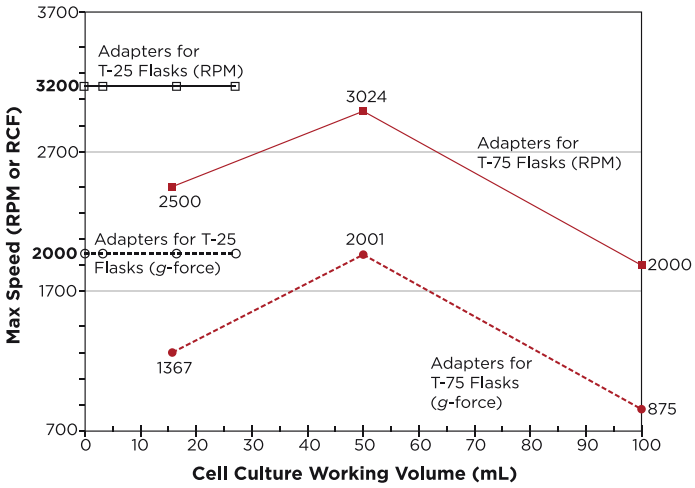
1. All centrifugation runs conducted at room temperature (18º C–25º C), during a cell passaging stage.
2. % cell PT = (cell in pellet) / (total cell) x 100; or % Recovery = (total cell after spin) / (total cell before spin) x 100.
3. Corning 75 cm² canted-neck cell culture flasks were used for the experiments.

Maximum Speed with the Cell Culture Flask Adapter

The maximum allowable centrifugal speeds of the Cell Culture Flask Adapter (rpm or *g*-force) are shown in Table 4. Maximum allowable speed varies according to working volume.

The Cell Culture Flask Adapter for T-75 flasks can be centrifuged up to 3,024 rpm (2,001 x *g*) with 50 mL (a very commonly used culture volume). Speed reduction is required for higher or lower working volumes. The adapters for T-25 cell culture flasks offer superior performance at 3,200 rpm (2,000 x *g*) up to 25 mL.

Table 4. Performance Range of Cell Culture Flasks for T-75 and T-25 Flasks**



Your Pellet Will Look Different

The difference in geometry between the flat bottom of the flask and the conical tip of the tube leads to a different appearance of the cell pellet. When centrifuging the flask directly, the cell pellet is softer and spreads more loosely over the bottom of the flask (Fig. 5).

Even though the appearance of the cell pellet is different, the results from centrifuging in the flask have been comparable to those from traditional methods.

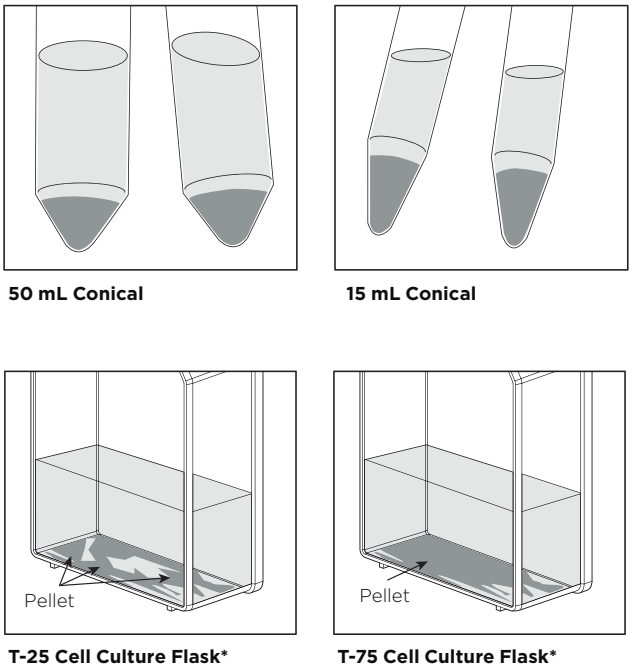


Figure 5. Different appearance of cell pellet obtained from tube or cell culture flask centrifugation.

**T-75: 75 cm² canted-neck cell culture flasks; T-25: 25 cm² canted-neck cell culture flasks.

Expect to Leave a Thin Layer of Media Above the Pellet

When centrifuging in a flask, the separation line between the cell pellet and supernatant is less clearly defined. After centrifugation, expect to retain a thin layer of residual media when removing the supernatant (Fig. 6). More spent media or washing buffer will be carried over to the next culture. This may result in diluting the strength of culturing nutrients. This phenomenon can be readily corrected with a smaller culture volume and increased centrifugal speed as described in Table 3.

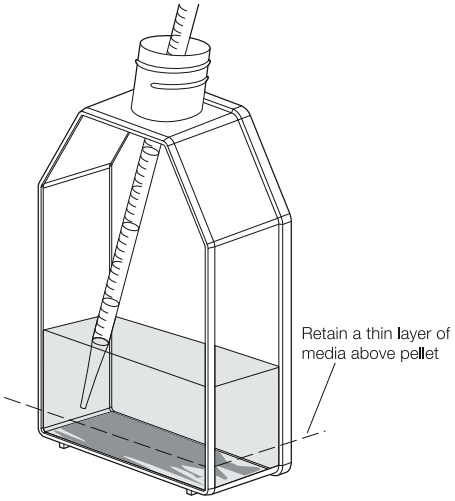


Figure 6. Residual media.

Major Benefits of Cell Culture Flask Adapters

— Streamlined cell culturing process.

— Time, labor, and labware savings.

— Reduced potential for contamination.

Additional Benefits from Cell Culture Flask Centrifugation

— Time and material savings multiply with the number of passages performed.

— Some small and less-dense cells seed better when centrifuged in the cell culture flask.

— Faster detachment of adherent cells during cell recovery.

— Softer cell pellets can be more readily resuspended.

Supply List

369292 Cell Culture Flask Adapter (set of 2), for Corning 75 cm² Canted-Neck Cell Culture Flasks (P/N 430641), includes 50 gram pack of dry release agent and two applicators

369295 Cell Culture Flask Adapter (set of 2), for Corning 25 cm² Canted-Neck Cell Culture Flasks (P/N 430639), includes 50 gram pack of dry release agent and two applicators

392819 Replacement pack dry release agent (200 grams), includes six applicators

*Beckman Coulter Cell Culture Flask Adapters have a patent pending.
†Adapters currently available for Corning 75 cm² canted-neck cell culture flasks (P/N 430641) and 25 cm² canted-neck cell culture flasks (P/N 430639).

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For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at www.beckmancoulter.com