

# Rapid Harvesting of High Volume *E.coli* Cells when using Thermo Scientific Contifuge Stratos Continuous Flow Centrifuge

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## KEY WORDS

- Table Top Continuous Flow Centrifuge
- Harvesting High Volume of Cells
- High Throughput
- Large Scale Cells

## Summary

In this applications brief, we describe an efficient method to harvest multiple liters of *Escherichia coli* (*E.coli*) cells using continuous flow centrifugation. Whereas a traditional fixed-angle rotor would require over four hours of harvest and setup time to process

over 18 liters of sample, the Thermo Scientific Contifuge Stratos tabletop continuous flow centrifuge performs this separation in **less than one hour**. For less batch-to-batch deviation and more consistency, it is often critical for *E.coli* cells to be harvested, then quickly processed or placed in frozen storage. Separation at this scale is clearly more efficient using the Contifuge Stratos centrifuge rather than batch mode centrifugation. The purpose of this study was to quickly and efficiently process *E.coli* from the culture medium using the Contifuge Stratos centrifuge.

## Introduction

*E.coli* is the most commonly used organism for protein production<sup>1,2</sup>. One of the reasons is that this organism is very well known and established in each laboratory. Also, its short generation time and resilient growth characteristics make *E.coli* suitable and extensively utilized host micro-organism for recombinant protein. Furthermore, pelleted bacteria can also be frozen, and later revived without any genetic change.

Most bacterial expression systems result in the collection of expressed protein in the cytoplasm (for soluble proteins) or inclusion bodies within the cytoplasm (for insoluble proteins). Others result in the collection of expressed protein in the periplasmic space, between the inner and outer membranes of the bacterium. It is rare to find a bacterially expressed protein

excreted into the growth medium. Thus, most bacterial expression systems require a centrifugation step to collect a pellet from which the expressed protein is purified. Centrifugation concentrates the expressed protein while removing potential contaminants.

Therefore, with time being a precious commodity, it has been demonstrated that the Contifuge Stratos centrifuge can efficiently separate over 18 liters of *E.coli* in under an hour.<sup>3</sup> This is in contrast to a normal batch centrifuge, which usually requires over four hours of processing and set-up time. The full capabilities of the Contifuge Stratos when using the continuous flow rotor are shown in Table 1.

## Materials and Methods

The *E.coli* cells were grown in two 10 liter B. Braun Biostat B fermentors at 25 °C under fed-batch conditions. The culture was induced by the addition of IPTG (Isopropyl β-D-1-thiogalactopyranoside) to a final concentration of 0.1 mM to produce a cytoplasmic recombinant protein. This protein is an essential *Bacillus subtilis* enzyme involved in biosynthesis of several co-factors. To obtain the desired high productivity, the culture was supplemented with pure oxygen. As a result, the fermentation achieved high cell density with a final optical density at 595 nm (OD) of 28, which is equivalent to 51.0 g/mL.

*E.coli* cells were centrifuged using the Thermo Scientific Contifuge Stratos tabletop centrifuge. With the continuous flow rotor *E.coli* cells were spun at a speed of 16,000 rpm (22,161 xg) and at an operating temperature of 4 °C. The harvest flow rate was ~ 450 mL/min and the total working volume of the two fermenters harvested was 18 L.



**Figure 1.** Thermo Scientific Contifuge Stratos and its continuous flow rotor (catalog no. 75003049) on cart with castors and pump secured in its compartment

## Contifuge Stratos Specifications

Max. Speed (rpm)	17,000
Max. RCF (xg)	25,040
Max./min. radius (cm)	7.75 / 5.00
Max. flow rate (mL/min)	600
Rotor mass (kg/lb)	5.7/13.0
Max. sediment volume (mL)	450
Material	Titanium

**Table 1.** Specifications of the Continuous Flow Rotor (cat. number 75003049)

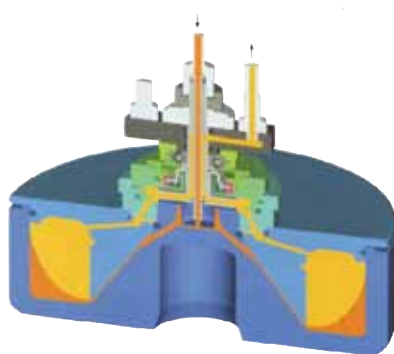


**Figure 2.** Thermo Scientific Contifuge Stratos continuous flow rotor (catalog no. 75003049)

## Results and Discussion

Depending on the starting sample volume, experience has shown that a culture with a final OD of 28 (51 g/mL) would require multiple runs for total separation. Knowing that the full sediment capacity of the continuous flow rotor was approximately 470 g, the culture was harvested in two centrifugation runs to avoid losing cells. After half of the culture was pumped through the Contifuge Stratos, a pellet weighing 448 g was collected and removed from the rotor.

The centrifuge was restarted and the remainder of the culture was processed. An additional 470.8 g of cell paste was collected. A total of 918.87 g wet cell weight *E.coli* was collected, with approximately 100 mL of residual liquid remaining in the rotor after the run was complete.



**Figure 3.** Cutaway Diagram of Contifuge Stratos continuous flow rotor (catalog no. 75003049)

Since the protein of interest was expressed in the cytoplasm, the pellet was saved and the supernatant was discarded. The consistency of the pellet was firm, which is very important, since a “loose” or watery pellet will give an inflated cell weight. Such inaccuracy can lead to erroneous productivity predictions. The cell pellet was frozen at -80 °C until utilized as raw material for protein purification.

The Contifuge Stratos centrifuge processed 18 liters of culture in under an hour. In contrast, a standard six-place batch rotor holding 500 mL bottles would require at least six 20 minute spins. Therefore, approximately four hours of processing time would be required for 18 liters of sample, especially when hands-on time is considered. In addition, because there are multiple runs, this can easily lead to batch-to-batch deviation and greater

inconsistency in the cells.

While batch centrifugation remains a widely used and popular method of separation, the Contifuge Stratos runs at maximum efficiency from the beginning, resulting in much higher throughput and less batch-to-batch deviation. Furthermore, fewer operator interventions are required with the Contifuge Stratos compared to a typical batch centrifuge.

## Other Applications

- Large volume sample processing in a single run
- Flow rate up to 36 L/hr
- Harvesting of Bacteria & Yeast; e.g. *Escherichia coli*, *Clostridium acetobutylicum* 792, *Acetobacterium woodii*, *Saccharomyces cerevisia*, *Bacillus subtilis*, etc
- Life science applications: Harvesting mammalian cells and insect cells, organelles, proteins, large viruses, nucleic acids, etc
- Non-life science applications: Separation of particles & sediments from clay, water purification, charcoal, paint, etc
- Typical process volumes of 20 - 80 liters depending on particle size and cell density
- Processes a maximum of up to 80 liters of suspension depending on sample density and particle size

For further details see table 2.

Sample	Particle Size (µm)	Flow Rate (mL/min)	Speed (rpm)	Degree of Clarification (%)	Comments
<i>Acetobacter woodii</i>	1 - 2	500	15,000	99	Difficult to sediment
<i>Methanosarcina barkeri</i>		200	15,000	99	Strictly anaerobic
<i>Sulfolobus spec.</i>	2 - 3	440	15,000	99	Thermophile
<i>Clostridium acetobutylicum</i>		500	15,000	to 99.7	Anaerobic OD 578nm = 3.6
<i>Saccharomyces cerevisiae</i>	3 - 5	500	8,000	98	
Insect Cells/Sf9 Virus culture	16	200	6,000	n.d.	Very sensitive cells, nearly complete separation with high vitale
<i>Hela-cells</i>	10	650	9,500	n.d.	90% vigorous
Animal cells undergone lysis (MKS and RNA virus residue)		400	10,000	95	
Macrophages from Cattle Lungs	7 - 20	760	4,100	n.d.	
Plankton	1 - 5	200	6,000	n.d.	
Suspended matter from River Spree water (heavy metal)	0.2 - 1	360	17,000	n.d.	

**Table 2.** Centrifuge parameters for different cell harvesting applications

## Conclusion

During the proteomic period, the growth in the use of recombinant protein has increased greatly in the recent years. Bacteria remain most attractive due to the low cost, rapid use and high productivity.

In this area of proteomics and high throughput, large scale cell processing must be optimized. The Thermo Scientific Contifuge Stratos tabletop continuous flow centrifuge therefore reduces processing time for high capacity. It efficiently harvests 18 liters of *E.coli* in less than one hour, a process typically requiring over four hours in a batch centrifuge. This allows you to save time and ensures batch-to-batch consistency and less deviation in processing.

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