

# CentriPAK BioProcess Container (BPC)

Effective applicability of the Thermo Scientific CentriPAK BioProcess Container in mammalian CHO-S cell culture separation when the pellet or when the supernatant is the product of interest

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## Introduction

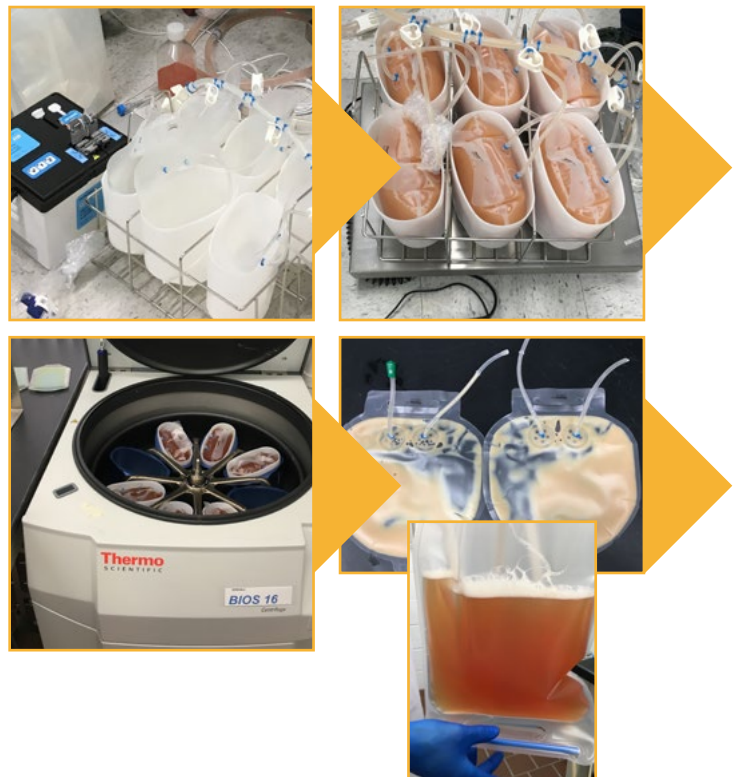
The following was undertaken to analyze cell viability post centrifugation in the cases where a customer would want viable cells for development of therapeutics, creating freezer stock seed or other purposes.

Batch centrifugation, causing no damage to fragile cells, is known as the preferred method for harvesting and concentrating cell due to low shear.

In the cell culture workflow for manufacturing biological products such as monoclonal antibodies, development of therapeutics, proteins or vaccines, among others, the purity of the supernatant harvested after the up-stream step is critical to process down-stream smoothly with minimum numbers of purification steps and maximizing efficiency and lifetime of columns and filters.

This application note describes the use of batch large-capacity centrifuge Thermo Scientific™ Sorvall™ BIOS 16 (Cat. No. 75007685) with 8 x 2000 mL rotor (Cat. No. 75003982), and CentriPAK™ BPC Adapter (Cat. No. 75003873), CentriPAK BPC (Cat. No. 75003880), CentriPAK™ Labtainer™ BPC (Cat. No. 75003883) to study the viability of the cells and the purity of the supernatant

harvested after mammalian CHO-S cell culture, comparing cell viability as well as cell concentration after the harvest and after the separation.



## Procedure

For cell viability testing post centrifuge a SUB 500L CHO culture expressing IGG came to conclusion at 22.6 million cells per mL at 79.6% viability the 6 May 2019.

1.7 L was immediately centrifuged at 5373 x g for 20 mins at 15 °C (no time given to cool before). Samples were checked for viability and cell count before, at start, middle, end, and re-suspended cell pellet to see how centrifugation removed cells from culture and if it affected viability.

Viable vs dead cell counts are taken automatically in the machine in three locations and the average is automatically determined (all values can be exported).

For supernatant purity, CHO-S clone grown in Dynamis Medium in 1000L SUB:

- Dynamis media contained 6mM glutamine and 0.1% anti-clumping agent.
- Cultures were seeded at 0.2E06 cells/mL.
- At time of harvested the final cell density was recorded.
- BPCs were filled to 1.7 L. Cell separation in Sorvall BIOS 16 centrifuge.
- Cell media suspension was distributed into 1.7 L CentriPAK BPC manifold.

- CentriPAK BPC were filled with about 1.7 L of CHO culture. The chambers were sealed off manifold, inserted into the adapter sleeves, weighed to find counter balancing 1.7 L chamber within 100 g, and loaded into the centrifuge. The BPCs were then centrifuged in Sorvall BIOS 16 centrifuge at 5373 x g for 15 mins at 10 °C. After centrifugation 1.7 L CentriPAK BPCs were loaded into and supernatant removed.
- For the supernatant cell/debris counts the same procedure was followed except the chambers were removed from filling manifold by zip-tie or weld off method.

Also other examples using same method but different vessel volume.

## Results

The viability was 76.5% with 18.2 x10<sup>6</sup> total cells counted mL for sample left at room temp from before centrifugation. After completion a sample was resuspended from off the top of the about 130g pellet and counted at 85.7% viable with 68.28x10<sup>6</sup> total cells per mL. Viability increased by 12%.

Other tests were showing results as follow:

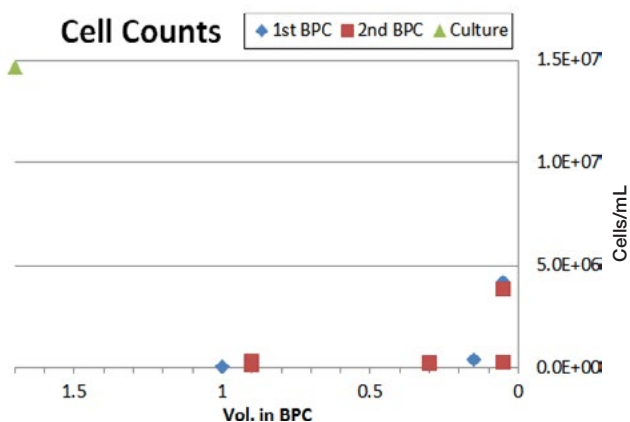
Viability increase 22%

Viability increase 21%

Table 1. Cell densities as measured with Countess II FL

| Date         | Vol (kg) | Culture Suspension |            | Supernatant debris leaving 1.7L BPC |            |  |
|--------------|----------|--------------------|------------|-------------------------------------|------------|--|
|              |          | Cell count before  | Start      | Middle                              | End        |  |
| 11 Oct. 2018 | 1.7 L    | 14,700,000/mL      | 29,300/mL  | 147,000/mL                          | 393,000/mL |  |
| 11 Oct. 2018 | 1.7 L    | 14,700,000/mL      | 176,000/mL | 158,000/mL                          | 199,000/mL |  |

Table 2. Cell count graph



Other example on 20 Nov. 2019

Sample from 250L CHO culture day after ending (chilled to 6C, nutrient limited): 10.7e<sup>6</sup> cells/ml,  
 CHO supernatant sample (2:1 dilution): 0.034e<sup>6</sup> cells/mL,  
 CHO resuspended pellet (10:1 dilution): 98.16e<sup>6</sup> cells/mL,  
 CHO resuspended pellet recheck (20:1 dilution): 120.49e<sup>6</sup> cells/mL

**Table 3. Cell densities as measured with ViCell**

| Date                         | Vol (kg) | Culture Suspension | Supernatant leaving 1.7L BPC |          |            | Notes                            |
|------------------------------|----------|--------------------|------------------------------|----------|------------|----------------------------------|
|                              |          | Cell count before  | Start                        | Middle   | End        |                                  |
| 15 Apr 2019<br>1.7L sample 1 | 1.7 L    | 7,500,000/mL       | 6,000/mL                     | 5,000/mL | 0/mL       | 84g left in BPC                  |
| 15 Apr 2019<br>1.7L sample 2 | 1.7 L    | 7,500,000/mL       | 5,000/mL                     | 6,000/mL | 260,000/mL | 45g left in BPC                  |
| 6 May 2019                   | 1.7 L    | 22,600,000/mL      | 43,000                       | 28,000   | 35,000     | ~110g pellet<br>130g left in BPC |

## Conclusions

### About cell viability

Apparently, centrifugation causes cells to appear more viable.

Interestingly in all cases that I have run samples viability goes up with centrifugation!

Batch large-capacity centrifuge Sorvall BIOS 16 with CentriPAK is suitable for harvesting and concentrating mammalian cell without compromising, nor damaging the cell.

### About supernatant purity

The cell suspension is reduced significantly by centrifugation in the CentriPAK. It was seen that if all but 40 mL was left in the 1.7 L BPC in addition to the pellet then the cell count in supernatant was insignificant. If more of the pellet is removed, more cells are likely to get into supernatant. Even if pellet is disturbed by mistake by user it is still a 3000 times reduction in cell count as seen in data.

The CentriPAK significantly reduces the number of filters needed (greatly reducing the time needed for filtration and the costs). If a customer desires to assure cell free with depth filter they can allocate a filter to meet their desires. Though we did find that the Sorvall BIOS 16 reduced the size of depth filter significantly.

It is recommend to interrupt flow when at about 50 mL so all BPCs can be drained fully to prevent the cells entering 10 L before the last 58 mL (combined 350 mL from the six 1.7 L CentriPAK) is collected.

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