

Filtration Trials with **SAFC EX-CELL® CD Hydrolysate Fusion**

Introduction

Following the launch of the EX-CELL CD Hydrolysate Fusion, SAFC conducted a study with partner company Sartorius Stedim Biotech. EX-CELL CD Hydrolysate Fusion is the first fully chemically defined media supplement of its kind in the industry.

The purpose of the study was to determine the throughput and biological activity of each filter using the EX-CELL CD Hydrolysate Fusion mixture. SAFC provided the chemicals, media and solutions, while Sartorius Stedim Biotech provided all filters and related laboratory equipment. Cell line CHO-IgG, a proprietary chinese hamster ovary clone expressing a recombinant antibody, was used in conjunction with EX-CELL CD Hydrolysate Fusion powder that was mixed according to manufacturer's directions at 20x concentrate. Membrane materials were filtered using 0.2 µm and 0.1 µm sterilizing grade filters.

In order to measure the cell growth and recombinant protein production, the cells were placed in suspension shaker flasks and used to seed experiments that were conducted in 50 mL (30 mL working volume) TPP Bioreactor tubes. Initial cell density was 200,000 viable cells/mL. The cells were tested using three unique filters at a time in the Multitron Incubator.

Case specifics

All testing was conducted at Sartorius Stedim Biotech's Application Laboratory using "unprotected" 0.2 µm and 0.1 µm filters, as well as "protected" 0.1 µm filters. In order to become protected filters, each of the 0.1 µm filters used as a protected filter went through an extra process using prefilters. The filtrate was collected from these prefilters and then filtered through Mycoplasma-retentive Sartopore 2 0.1 µm PES filter at 5 psi differential pressure.

The filters used in all tests follow:

- Sartopore 2 0.2/0.1
- Sartopore 2 0.45/0.2
- Sartopore 2 XLG 0.8/0.2
- Sartopore 2 XLI 0.35/0.2
- Competitor A double layer 0.2 PES
- Competitor B double layer 0.2 PES

The prefilters used for the "protected" 0.1 µm filters follow:

- Sartoguard PES 0.1 µm nominal
- Sartoguard GF1 0.1 µm nominal
- Sartoguard PP1 0.1 µm nominal

Multiple run testing was not available for the 0.2 µm filters due to the high throughput they exhibited. Only Sartopore 2 XLI and XLG allowed for duplicate results. All other reports are based on a single set of test data.

Test outcomes

Overall, the EX-CELL CD Hydrolysate Fusion was easily filtered. Average throughput values ranged from about 10,000 to 60,000 L/m² for the unprotected 0.2 µm filters. In some cases, 3 L volume of the solution was not enough to display the accurate flow decay; the data in these cases was discarded.

Outcomes of the filtration studies follow:

- Highest average throughput: Sartopore 2 XLG 0.8/0.2 with 58,487 L/m²
- Closely following the XLG: Sartopore 2 XLI with about 35,000 L/m²
- All other filters: ranged from 10,000 to 30,000 L/m²
- Lowest average throughput: Sartopore 2 0.1 µm with 3,432 L/m²
- Best performing filter flux: Competitor B PES

“Unprotected” conclusion

Scale-up selection for EX-CELL® CD Hydrolysate Fusion filtration is heavily driven by flow rate requirements. In the studies conducted, flow rates ranged from 25% to 70% of the total area requirement.

Among the filters tested, the two best performers, Sartopore 2 XLG for throughput and Competitor B PES for flux, would require only one 10 inch element to filter 4,000 L of EX-CELL CD Hydrolysate Fusion in one hour. Less than one 10 inch cartridge is needed for filtration volumes under 4,000 L when processing at one hour at 10 psid.

“Protected” conclusion

Testing conducted on the Sartorius Stedim Biotech prefilters evaluated the protection that a prefilter provided to the 0.1 µm filters. Three prefilters were used at 10 psi in the evaluation: Sartoguard PES, Sartoguard PP1, and Sartoguard GF1.

- Sartoguard PES – provided the highest initial flux at 10,520 LMH
- Sartoguard PP1 – provided the highest throughput at 15,163 L/m²

The filtrate was collected after each test and pooled into a Sartopore 2 0.1 µm SartoScale at 5 psi to do the final test of the protection provided by the 0.1 µm filter.

- Sartoguard GF1 – provided the highest throughput at 39,360 L/m² and highest flux at 1,016 LMH

Growth curve testing

Growth curve testing using a CHO cell line was conducted to clarify whether performance differences could have been caused by the different filter trains. Based on the growth data collected, all filters produced nearly identical results. Similar results were seen when comparing IgG productivity profiles.

Final conclusion

Overall, the testing has identified the prime filter candidates for verification trials with small pleated devices. The lack of performance difference suggests that filtration of the EX-CELL CD Hydrolysate Fusion can easily be transferred to 0.1 µm filters to gain further sterility confidence, if needed for the specific process.

Important observations from testing:

- Pre-filter would not be needed for 0.2 µm filtration of large-scale commercial batches.
- Sartopore 2 XLG 0.8/0.2 performed the best among the 0.2 µm filters.
- Sartoguard PPI had highest overall individual throughput and Sartoguard PES had highest initial flux among the prefilters. However, Sartoguard GF1 offered the best protection of Sartopore 0.1 µm.
- A prefilter would most likely be needed for batches 2,500 L and above and/or for processing times of less than one hour for 0.1 µm terminal filtration.
- More trials should be conducted to obtain repeatable results. Flat disc results need to be verified for running trials on small pleated devices for all filters, as well. Different media could also be tested to see if they influence variability in throughput.
- EX-CELL CD Hydrolysate Fusion is supplied as a 20x liquid (Catalog No. 14700C) or dry powder (Catalog No. 24700C). It can be used as an alternative to hydrolysates in any part of a CHO cell culture process. It also has applications in other cell lines, such as NS0 and SP2/0.
- Utilization of 0.1 µm terminal filter trains does not affect cell culture performance of EX-CELL CD Hydrolysate Fusion when compared to standard 0.2 µm filter trains. This can allow for increased flexibility and sterility confidence.

Learn more about
CD Hydrolysate Fusion

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