HarvestMax 1000: A Single Step Sterile Filtration of CHO Cell Cultures Producing Therapeutic Proteins

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Introduction

With the improvements in CHO cell productivities, milligrams to gram amounts of therapeutic protein can be produced in a standard shaker flask with working volume of 30mL to 1L. However, traditional harvest clarification methods necessitate scientists to rely on time-consuming centrifugation to separate cells from the conditioned media containing protein of interest. The complexity of the process increases further as the culture volume increases to liter scale. Typically, the clarified supernatant is then sterile filtered through 0.2uM flat disc membrane filters before the downstream purification can be initiated.

Marin Scientific has developed HarvestMax 1000 (Figure1), a single step filtration device that combines a nominal 1.2 µm pre-filter mounted on top of 0.2 µm vacuum filter reservoir. The pleated pre-filter offers massive surface area with unlimited capacity for removal of cell debris and macro-particulate load. As a result, mammalian cell culture harvest can be poured directly from the flask or bioreactor into the HarvestMax 1000 reservoir without a centrifuge clarification step. The negative pressure generated by the standard laboratory vacuum device is sufficient to pull through clarified conditioned media through the 1.2 µm NGF pre-filter, and subsequently through the 0.2 µm PES membrane filter into the receiver bottle as sterile filtrate.

In this application note, we will describe the optimized workflow and accessories that has yielded 90- 95% v/v of the sterile filtered conditioned media from CHO cell culture harvest in a matter of minutes.

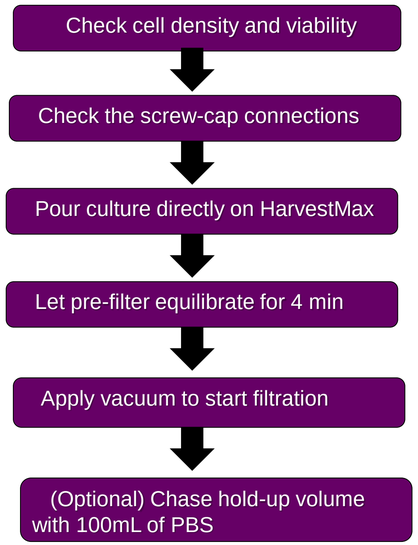
Material and Method

Cell Culture
A CHO-S stable pool generated by puromycin selection expressing ScFv molecule is used in this study. The production media is a serum free formulation, custom made for CHO cell line. The cells were seeded at 0.8E6 cells/mL at a working volume of 2L in a 5L Erlenmeyer shake flask. The culture temperature was lowered from 37C to 31C on day5. The culture was supplemented with 10% nutrient feed on day 4 and harvested on day 7.

Filtration device
HarvestMax1000 filter assembly was used either with or without pre-wetting by150mL PBS before pouring the cell culture harvest on the pre-filtration unit.

Cell Counts
Vi-cell automated cell counter was used to monitor the cell density of the CHO cell culture

Optimized Process Workflow



Notes

- Cell density range: 1E6/mL – 20E6/mL
- Viability: 20% - 99%
- Cell type: CHO, 293, Hybridoma Cells

Figure1 HarvestMax 1000 for single step sterile filtration

Figure2 Pleated 1.2 uM Glass microfiber with void displacer unit to minimize hold up and improve recovery

Facile Set up for HarvestMax1000

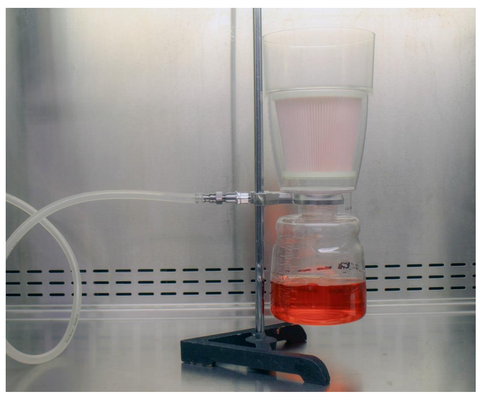


Figure 3
HarvestMax is a single-step filtration device providing sterile filtered conditioned media from cell culture harvests without a need of time consuming centrifugation step

Results

No pre-wetting of 1.2uM pre-filter is required to achieve higher recovery from HarvestMax 1000

Filtration Run 1 With Pre-Wetting
CHO Material: Viable Cell Density @ 13.4e6/mL and 95% viable (Total volume = 1,040 mL)
Customer poured 150 mL of PBS into HarvestMax reservoir to soak the pre-filter for a few seconds before dumping out the PBS buffer into the sink. After 18' of applied vacuum pressure, 960 mL of filtrate was recovered (~8% hold-up volume) in the receiver bottle. No chase buffer was used.

Filtration Run 2 Without Pre-Wetting
CHO Material: Viable Cell Density @ 13.4e6/mL and 95% viable (Total volume = 1,075 mL)
Customer poured the production broth directly into the HarvestMax reservoir. After 18' of applied vacuum pressure, approximately 950 mL was recovered in the filtrate (~12% hold-up volume). Then customer poured 150 mL of PBS to chase the hold-up volume into the filtrate. A final total volume of 1,050 mL was recovered after chase.

No loss of titers is observed in antibody variant producing CHO culture with HarvestMax 1000

Either low titer (less than 50 mg/L) or high titer (400 – 1000 mg/L) CHO production cultures were filtered using HarvestMax 1000. The titer of the final filtrate samples and centrifuged harvest were measured using Octet Red instrument using ProA biosensors. No loss of titers were observed in any of the filtered cultures indicating that HarvestMax can efficiently filter the CHO cell culture in single step without any loss of titers in the process.

Summary

- ❑ HarvestMax is a single-step filtration device providing sterile filtered conditioned media from cell culture harvests without a need of time consuming centrifugation step.
- ❑ We have outlined an optimized workflow and accessories that result in high recoveries of over 95% with minimum hold up volume
- ❑ The filter unit has been tested for a variety of cell types including CHO, HEK293, hybridoma cells.
- ❑ No loss of titers during the filtration was observed using HarvestMax 1000 for low or high titer cultures

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