

New Single-Step harvest process that helps you save time and money in early to late stage cell culture research and development. Recovering more of your valuable cell culture derivatives and improving yield.

HarvestMax 1000 Technical Bulletin

Technical Services



MARIN SCIENTIFIC
DEVELOPMENT COMPANY





HarvestMax 1000



Introduction

HarvestMax 1000 combines superior pre-filtration performance and ease of use compared to common laboratory filtration methods for mammalian cell culture harvest.

Traditionally, there have been two ways to perform membrane filtration to separate conditioned media from macromolecule debris in research scale mammalian suspension cell cultures of 0.5 – 5 liters:

- 1) Single flat disc filter membrane (pore size 0.1 - 0.5 μm absolute) mounted on a reservoir/receiver unit;
- 2) Combination membrane filters housed in a polypropylene capsule, also known as capsule filters (pore size 0.5 - 3.0 μm nominal or absolute).

Both filtration methods require product clarification via a centrifugation step of between 20 to 60 minutes at 3,000 to 4,000 RCF before the supernatant can be either loaded onto the filter reservoir, or pumped into a capsule filter. For larger scale productions, depth filters have been used to clarify the culture harvest in lieu of centrifugation before sterile membrane filtration. Depth filtration has severe drawbacks for the research scale i.e. low throughput, high holdup and/or high cost.

Flat disc membranes, such as the ones found in laboratory vacuum filter units, have an absolute cut-off and are widely used. However, because the surface area of the filter membrane is quite small ($\sim 64 \text{ cm}^2$ for a 90 mm disc), the filter may quickly foul when loaded with typical 6 – 14 days old mammalian cell broth from fed-batch processes, even after the macromolecular debris has been removed by centrifugation.

Capsule filters offer reduced risk of membrane fouling if flowed slowly i.e. 30 -50ml/min. due to multiple pre-filter and membrane sheets layered inside the capsule housing to provide up to $\sim 0.13 \text{ m}^2$ of filtration area. The sheets may be of the same material such as glass microfiber or PVDF, or a combination of materials. There is a significant risk of product holdup inside capsules, so actual holdup determination is recommended. Also capsules require a pump since they must be pressure driven. While the larger surface area improves particle load capacity compared to flat disc membranes, the filtrate from capsule filters is typically not sterile because;

- A) capsules require the use of a pump and the tubing connections and path are not sterile;
- B) the filter media is usually no better than 0.5 μm nominal, rather than absolute, rating.

In the typical harvest workflow, the filtrate from a capsule filter needs to be filtered again in a 0.2 μm absolute rated sterile filter before downstream purification or long term storage.

The HarvestMax 1000 filter unit combines the best of both worlds by incorporating a nominal 1.0 μm non-binded glass microfiber pleated sheet pre-filter mounted on top of a regular 0.2 μm sterilizing-grade disc membrane. The pleated pre-filter offers massive intrinsic surface area for significantly enhanced clarification capacity for cell debris and macro-particulate load. As a result, mammalian suspension 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 generator is sufficient to pull clarified conditioned media through the 1.0 μm pre-filter, and then subsequently through the 0.2 μm membrane filter into the receiver bottle as sterile filtrate.

The implementation of the HarvestMax 1000 filter into your laboratory process will save both money and time, while providing superior, sterile clarified product that is ready for downstream processes.



Pilot study using HarvestMax 1000 by a Large Pharmaceutical Company Customer

A fed batch process was initiated from a CHO-S suspension cell stable pool generated by Puromycin selection expressing a scFv molecule. The cell culture media was a serum-free supplemented custom media for CHO. The production was initially seeded at 0.8e6 cells/mL in a total volume of 2 L in a 5 L Corning™ Erlenmeyer shaker flask. The incubator temperature was lowered from 37°C to 31°C on Day 5. The culture was supplemented with a 10% custom feed on Day 4 and terminated on Day 7.

Filtration Run 1 With Pre-Wetting

CHO Harvest: 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 402mmHg 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 Harvest: Viable Cell Density @ 13.4e6/mL and 95% viable (Total volume = 1,075 mL)

Customer poured the harvest broth directly into the HarvestMax reservoir. After 402mmHg of applied vacuum pressure, approximately 950 mL was recovered in the filtrate (~12% hold-up volume). Subsequently 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.

Summary of HarvestMax 1000 Advantages Over Pump Driven Harvest Methods:

- Clarification is faster than centrifugation without the capital and maintenance costs
- Ease of user operation and lower overall operating costs
- Users are not tied to any particular proprietary flask and/or transfer cap
- Reduced contamination risk and waste by eliminating need for pump tubing
- Reduced plastic waste compared to traditional filter units
- Eliminate use of peristaltic pumps
- Reduced holdup as compared with capsule filters
- HarvestMax is often faster than capsule filtration: <10min.
- Vacuum manifold option allows higher throughput compared to single channel pumps
- Potential to eliminate need for centrifuging



FAQs

How Much Harvest Can I Filter Using a HarvestMax 1000?

Up to 1 – 2 liters of uncentrifuged 293 conditioned media can be filtered by a single HarvestMax 1000 unit (you will need an additional 1L receiver bottle for 2L of starting material). For CHO-derived material, the HarvestMax 1000 unit can process between 0.8 to 1.0 liters of uncentrifuged conditioned media. Hybridoma-derived material will show filtration performance similar to CHO material. Biological media is complex and it is difficult to predict how much media you can process before the sterile membrane will foul in every circumstance.

What Should I Do if the HarvestMax 1000 Fouls Prematurely?

If pre-mature fouling were to occur, the 0.2 um sterilizing membrane in the vacuum filter is typically the culprit that causes slowing. Disconnect the vacuum source and allow the space between the HarvestMax and the membrane on the filter unit to equilibrate may help recover flow. Or it might be helped by momentarily shutting the vacuum off. If not, you can always replace the HarvestMax unit too and continue filtering the rest of the material.

How Do I Improve Flow Rate and Yield?

In general, lower cell density and lower cell death will tend to yield more volume of filtered media and a higher filtrate flow rate per HarvestMax unit. Increasing the vacuum pressure will help increase filtrate flow rate. Twirling or tilting the HarvestMax Pre-Filter unit like a glass of wine helps to speed up the prefiltration by leveraging the entire surface area, but it will not help if the sterile 0.2 µm membrane filter on the vacuum filter unit has fouled. In this case use of the optional HarvestMax Pre-Filter Spacer Accessory (Cat. No. HMS-100) to occupy upstream volume may improve recovery.

How Do I Reduce Hold-Up (Dead) Volume?

Chase the hold-up volume from the prefilter by pouring in an additional volume of buffer equal to the hold-up volume (i.e. 100 to 120 mL). Twirl the unit around to ensure good mixing, then continue to apply negative vacuum pressure until you are done.

Please note that the chase buffer will dilute your product, but not by much, because the chase buffer volume will be mixed with the ~10% residual product on the prefilter. In other words, your final product volume will be 95% product and 5% buffer after a 10% chase.

Prewetting the filter unit with buffer is not recommended due to dilution of the product without reducing hold-up volume. Prewetting with harvest is OK and recommended.



Cost Analysis for a Typical 1 L Fed Batch Production

Non-HarvestMax Process

1 x Whatman G.E. Polycap HD Capsule Filter (\$100) and pump, 30 min.;

or 15min. – 20min. centrifugation (FTE \$50 -\$75)

1 x Nalgene Mach V 0.2 um mPES 1 L reservoir vacuum filter + receiver bottle (\$15);

2 x single use disposable 500 mL PP centrifuge tubes (\$10);

1.0 FTE/hr (\$100); + whatever cost can be attributed to degradation of protein

Total cost = \$175 (excluding capital cost of centrifuge equipment and service contracts)

HarvestMax 1000 Process

1 x HarvestMax 1000 unit

0.3 FTE/hr (\$15)

Total Time = 5 – 8 minutes



Sample Request Questionnaire

To receive a free sample, please fill out your application details and agree to anonymously share your trial Data, and we will send you a free sample to test.

1. Please let us know how you presently accomplish this task of small volume clarification i.e. centrifugation, filtration with vacuum filters in series, capsules hollow fibers or other.

2. What is your starting cell concentration and cell type i.e. BHK, CHO, Hybridoma, 293, Transfected etc.?

3. In what order of importance do you place the following in this step? Low, Med or High? Cost _____ Ease of use _____ Time Savings _____ Space Savings _____ Recovery: _____
4. How would you classify your organization? Biotech or Pharmaceutical Company, CMO, CRO, University, Medical Research Center, Government NIH, etc. (please circle) or other: _____

Name: _____

Organization: _____ Mailing Address: _____

Phone: _____ Email: _____ UPS / Fed Ex Acct. _____ (optional)

Please check the box closest to your application or Dept.:

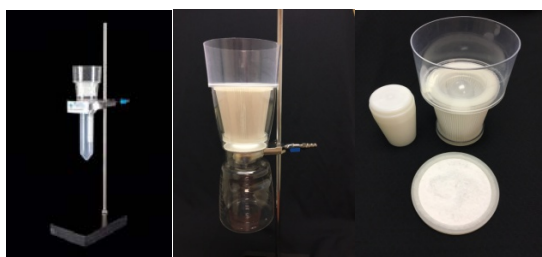
- Therapeutics Development
- Early or Late Stage Cell Culture Development
- cGMP Pilot Plant or Production
- Plasmid DNA Purification
- Protein Production (in bacterial, mammalian, or insect cell systems)
- Antibody Manufacturing (vectors, proteins, or antibodies)
- Gene Synthesis
- Genomic DNA Production
- Host cell optimization
- Expression screening (cells, media, construct, culturing conditions)
- Process development
- High Throughput Cell Culture Development
- Custom Monoclonal Antibodies
- Enzyme and antibody assay design

Please scan or copy and email your request to: info@marinscientific.com



Harvest-Max™ 15, 50, 250, 500, 1000

FILTER RESERVOIR SYSTEM FOR ENHANCING FILTRATION TOTAL THROUGHPUT OF VACUUM FILTER UNITS. PATENT PENDING.



HarvestMax™ filter inserts work with our 15ml (not shown), 50ml (left), 250ml and 1000ml (middle) VACU-Max filter units. The convenient accessory spacer, shown in the far right image, reduces upstream void volume & hold up loss in the 1000ml unit. Triple-Layer Coarse Cotton Ring Filter is shown in bottom of above right side picture.

Description

Harvest-Max™ filter accessory is part of a family of single-use, drop-in prefilter units used for the prefiltration clarification and final filtration of difficult to filter cell culture harvests, conditioned media, microbiological media, and other biological fluids and chemical reagents. It represents the first true 30ml to 1L 1-step processing. The filter is designed to significantly reduce the risk of premature plugging of standard 1L and smaller vacuum membrane filter units. More importantly it reduces harvest clarification time and eliminates the need for centrifugation. No more need to plug multiple vacuum filter units or use costly capsule filters. Reduction of FTE and reduction of your proteins exposure to proteases are a significant benefit. Not to mention laboratory floor space savings when the centrifuges are gone!

The Harvest-Max™ family of special filters are available as complete vacuum filter systems in stock and ready to use. They can also be customized for your particular application.



Filter Media: Inert binder free depth media

Support Materials: End Cap & Reservoir: Styrolux
Potting: Synthetic Polymer Hot Melt

Sterilization: Irradiateable, GAMMA

Capacity: 15ml, 50ml, 250ml, 500ml, 1000ml

Temperature range: -20°C to + 40°C

Applications:

Antibody preps, CHO-Hybridoma-293 & HEK whole harvests, conditioned media or buffers, biological preparations, Reagents, Prefiltration of sterile media feeds, Antifoam additions, Transfected cells, Crop Protection - Bacterial harvests, Food Digestion Preparations, Protein solutions and viral, vaccine or tissue preps.



3 place manifold:

Features:

- Eliminates need for centrifugation and allows manifolding of many units for simultaneous filtration.
- More prefiltration filter area allows for faster flow and higher throughput through the membrane filter.
- Utilizes convenient quick-connecting Vacuum-Cradle ring stand clamp.
- Universal design can be used with most 1L vacuum filter units commonly found in the laboratory.
- Vacuum unit membranes from 37mm & 90mm diameter, PES membrane and are available in 0.1µm, 0.2µm or 0.45µm Nominal 10µm coarse or gradient density prefilter media are available for targeted applications.

Part Number	Description	Pricing
HM-88021	15ml - 0.2µm PES	\$TBD
HM-88031	50 ml - 0.2µm PES	\$TBD
HM-88052	250 ml - 0.2µm PES	\$169.00
HM-88055	500ml - 0.2 µm PES	\$179.00
HM-88051	1000ml - 0.2µm PES	\$195.00
HM-88000	Vacuum-Cradle Clamp	\$202.15
HM-88002	Upstream Hold-Up Spacer	\$179.00
HM-88003	Coarse Media Prefilter Ring 3-PK	\$35.00
HM-88004	3 Place Vacuum Manifold, AL/ S.S.	\$1,299.00
HM-81001	HarvestMax C1000 w/gasket for use with other vacuum units.	Call for Quote

