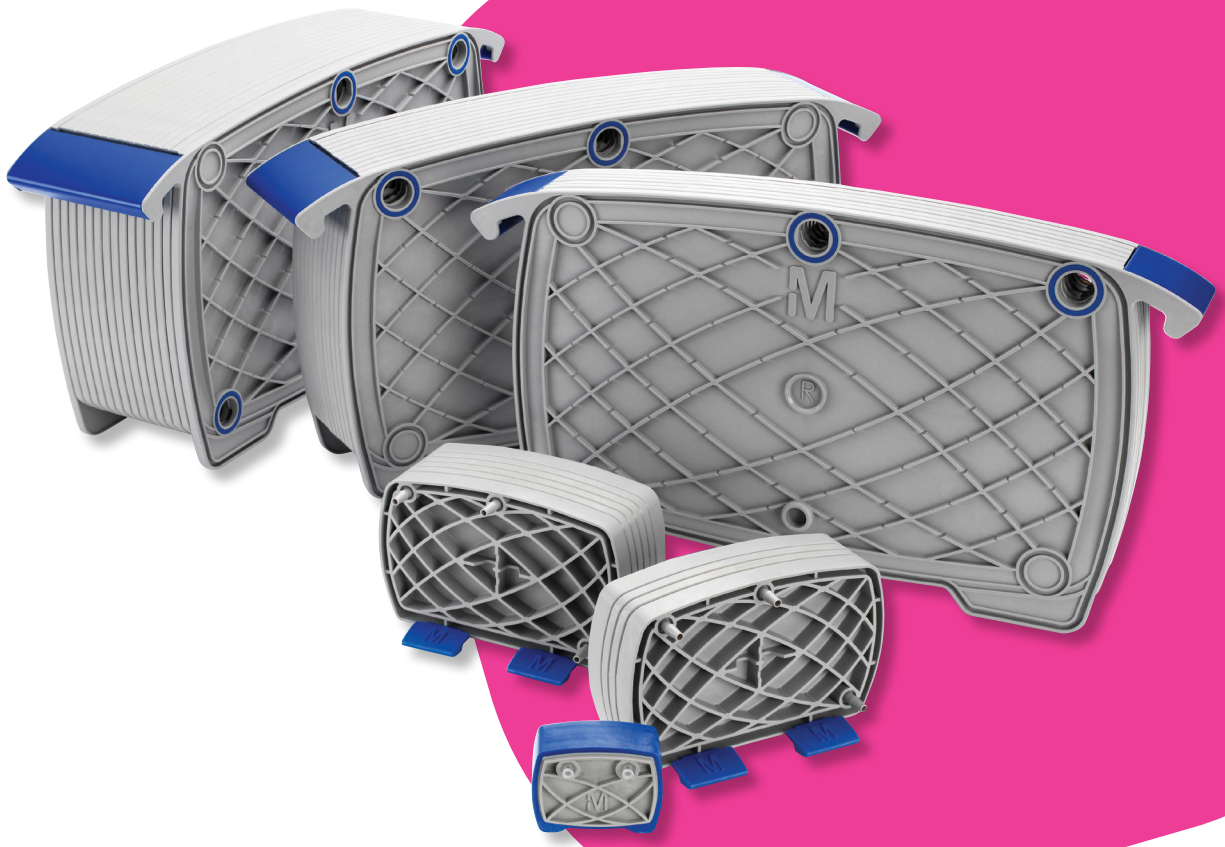
Pmax™/Tmax™ Constant Flow Rate Test for Depth Filter Sizing



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Introduction

The constant flow rate Pmax™/Tmax™ approach can be performed on all types of filters including membrane filters, surface filters and depth filters. However, as it does not assume any underlying mechanistic model of filter plugging, it is commonly used to size depth filters and other charged filters that exhibit complex fouling models where particle retention occurs via size exclusion and adsorption.

This protocol describes small-scale depth filtration studies that are performed on bioreactor harvest material, pre-processed harvest material (e.g. centrate) or process intermediates in order to determine the capacity of the depth filter and to size filters for larger scale operations. Pre-treated bioreactor harvest clarification (acid precipitation, polymer pre-treatment, etc.) is not included as part of this protocol.

This protocol has been designed for performing a sizing trial using Millistak+® depth filter in µPod® format.

Technical Assistance

For more information or questions, please contact: **EMDMillipore.com/techservice**

Material

1.1 Feed stream

Fresh and representative feed stream must be used for small-scale study (temperature, cell density, cell viability, age of the feed).

Water for injection (WFI) and buffer will also need to be used for depth filter preparation and equilibration prior to product depth filtration.

1.2 Filtration devices

Filtration devices, in µPod® filter format (23cm²) for:

Clarification	Depth filter grade	Depth filter material	Catalogue No.
Millistak+® HC depth filter			
One-stage clarification	C0HC	Cellulose positively charged depth filters with diatomaceous earth filter aid material	MC0HC23CL3
Two-stage clarification	D0HC		MD0HC23CL3
	X0HC		MX0HC23CL3
Millistak+® HC Pro depth filter			
One-stage clarification	C0SP	Polyacrylic positively charged depth filter with silica filter aid material	MC0SP23CL3
Two-stage clarification	D0SP		MD0SP23CL3
	X0SP		MX0SP23CL3

Millistak+® HC and Millistak+® HC Pro filters are graded density depth filters with a wide range of nominal pore ratings (Figure 1):

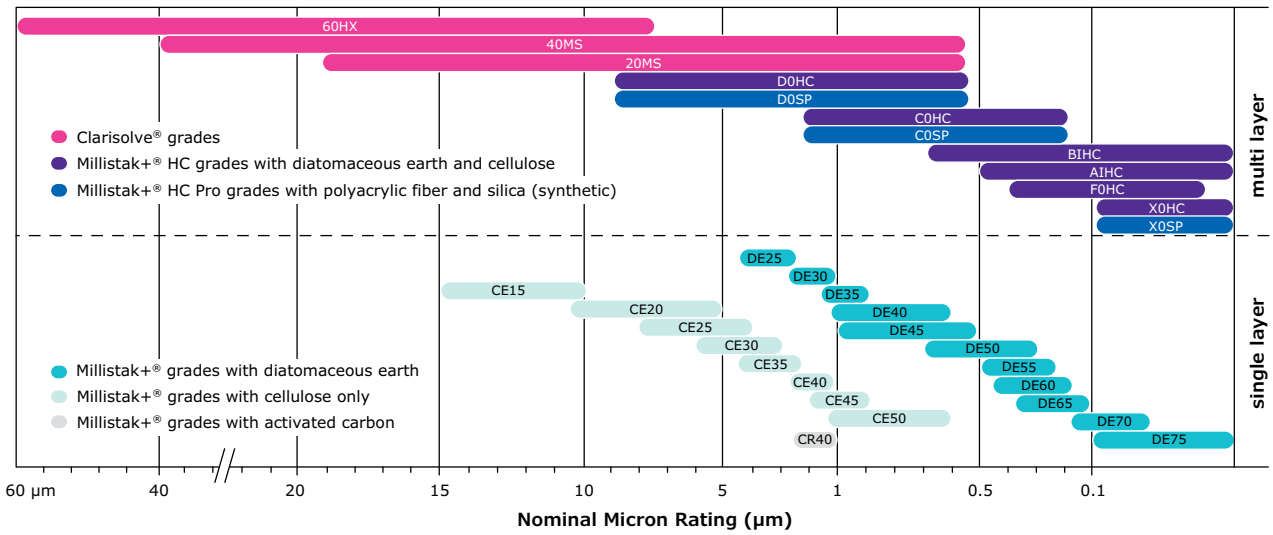


Figure 1. Porosities of Millistak+® HC and Millistak+® HC Pro filter grades

To select the right depth filtration for your process, please refer to the Selection Guide (PB1421EN00) or contact us.

1.3 Equipment

The following equipment will be required for the trial:

- A peristatic pump/positive displacement pump
- One or two pressure gauges with Luer lock connection
- One roll of tubing (ideally Masterflex® with diameter 16)
- Female and male Luer connections for tubing (Valve Plastics, Inc., Part Number MTLL230-1 and FTLL230-1)
- Two Luer valves per depth filter (Cole-Parmer® Catalog Number WU-30600-00)
- Two three-way Luer valves for two stage depth filtration only (Cole-Parmer® Catalog Number WU-30600-02)
- A timer
- A turbidity meter and vials
- Disposable pipettes and one pipette controller
- A balance (0.1 g accuracy)
- A stir plate and bar to gently mix product during the test
- Beakers and bottles to collect filtrate and vent output during trials
- Personal Protective Equipment (PPE): safety glasses, gloves and lab coat
- Printed copy of data collection sheet

1.4 Bench space utilities

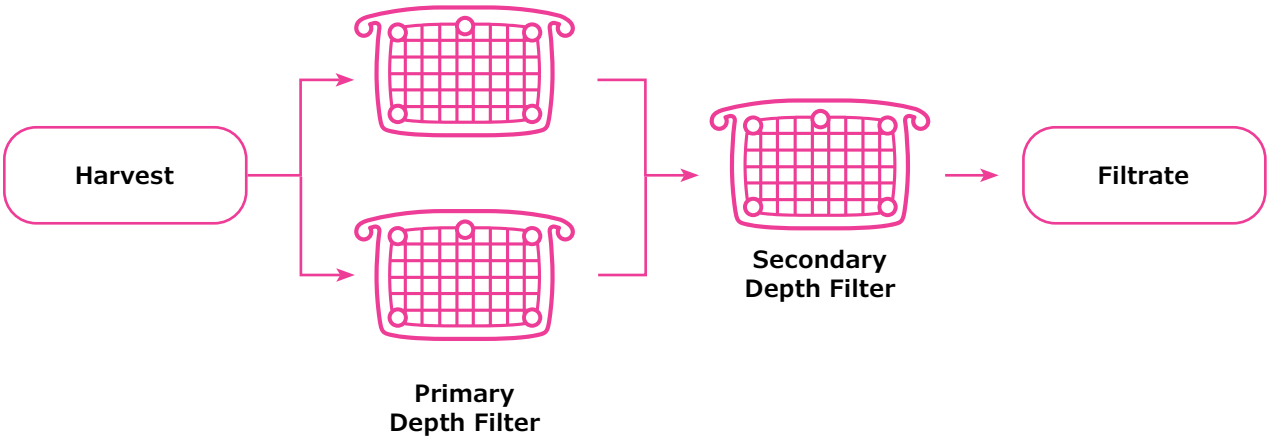
The following utilities will be required for the trial:

- 2 m linear bench space
- Drain
- Electricity supply

Methods

The method below should be used in conjunction with the µPod® Filters User Guide. Familiarize yourself with the specifics of the method before you begin.

- Ensure that the equipment is clean before starting.
- Prepare your data collection sheet prior to the trials (Table 1, page 8).
- If the process requires two clarification steps (a primary clarification followed by secondary clarification), depth filters must be assessed in-line. In-line testing must be done with the appropriate filtration area ratio between the primary and secondary depth filters to mimic dynamic plugging and pressure distribution across the filtration train. If no information about the area ratio is known, a 2:1 ratio between primary and secondary depth filter should be tested:



- At the end of the Pmax™/Tmax™ experiment, collect the filtrate and assess the quality of the prefiltered material through a sterilizing-grade filter (Millipore Express® SHC 0.5/0.2 µm membrane in an OptiScale® 25 filter format is recommended, ref SHGEA25NB6). This testing should be performed according to Vmax™ test procedure. Please refer to Vmax™ Aseptic Protocol (BR3860EN).
- Note that a Vmax™ value over 5000 L/m² means that the product is non-plugging and well clarified. Vmax™ values around 200–5000 L/m² are considered moderately plugging and below 200 L/m² are considered highly plugging streams where insufficient clarification has been achieved.

2.1 Equipment set up

- Calibrate the pump if necessary.
- Assemble the filtration lines.
- Close all system valves.

2.2 Depth filter wetting with WFI

1. Fill a vessel with a known volume of WFI. Place inlet/feed tubing in the vessel (600 mL per filter is enough, tare the weight of the vessel before starting).

   Start the flushing process using the steps listed below and once the filter and tubing are fully filled with water, perform a mass balance to determine the hold-up volume.

2. Close downstream filter valve (b, b1, b2, b3).
3. Open all vent valves (a, a1, a2, a3).
4. Remove air from filters by starting to pump WFI at a flux of 600 LMH (i.e., 23 mL/min for one-stage clarification and 46 mL/min for two-stage clarification). When liquid is expressed from the vent valve (a, a1, a2), wait at least 10 seconds to ensure the filter is properly purged.
5. Open downstream valve (b, b1, b2), close vent valve (a, a1, a2).

   If working with two-stage clarification, repeat the operations (step 4 and 5) with the secondary depth filter using valves a3 and b3.

6. Turn the pump off once fluid starts dripping from the outlet.
7. Calculate the hold-up volume by measuring the volume of water remaining in the initial vessel of water, adding any water collected from the vent and outlet lines, and then subtracting from the initial known volume of water.
8. Restart the pump and flush filters for 10 min (to waste).
9. Stop the pump.

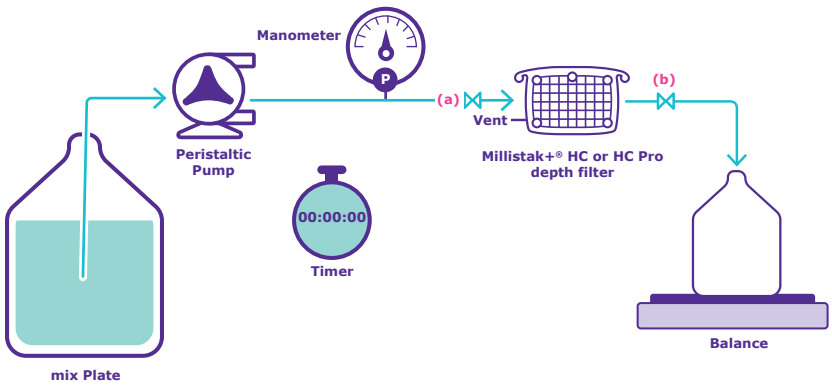


Figure 2.
One-stage clarification

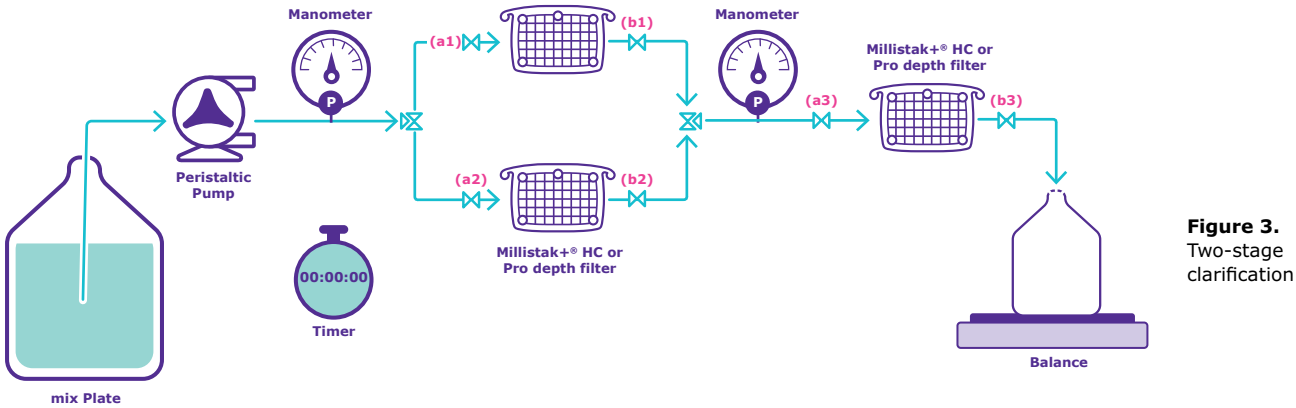


Figure 3.
Two-stage clarification

2.3 Buffer equilibration

1. Transfer the inlet tubing from water to buffer. Avoid introducing air into the tubing.
2. Restart the pump at the same flow rate as WFI wetting and continue to flush with buffer for approximately 3–4 minutes.
3. Stop the pump.

2.4 Product filtration

1. Place the product on the stir plate and ensure it is adequately mixing during the study.
2. Transfer the inlet tubing from buffer to product. Avoid introducing air into the tubing.
3. Measure the initial turbidity of the product stream using the turbidity meter.
4. Move the outlet tubing to a new or cleaned vessel placed on a balance. Tare the balance.
5. Set the pump to a process flux of 100-150 LMH (i.e., 4–6 mL/min for one-stage clarification, 8–12 mL/min for two-stage clarification).
6. Start the pump and the timer. The holdup volume retained in the system can either be collected if you are measuring product recovery with the product (be sure to account for dilution at the end) or separately and discarded. Use the Pmax™/ Tmax™ data collection sheet (see table 1 on the next page).
7. Record filtrate volume and pressure as a function of time every 2 minutes. Record turbidity as a function of time every 10 minutes.
8. When the upstream pressure has reached 22 psi/1.5 bar or when the online filtrate turbidity increases.
9. Measure and record the turbidity of the filtrate pool at the end of the experiment.
10. Move the inlet tubing to a known volume of buffer (equal to at least one system hold-up volume) for a post-process buffer flush (or recovery flush).
11. Start the pump at the process flowrate and continue filtering to collect a volume equal to the holdup volume. This should be done in a different collection vessel, if possible.
12. Stop the pump.
13. Release system pressure by opening filter vent valve. Disassemble and clean the system. Discard filters.
14. Analyze the filtrate and buffer flush samples for product to calculate yield/ recovery and impurity removal (HCP, DNA, etc.).

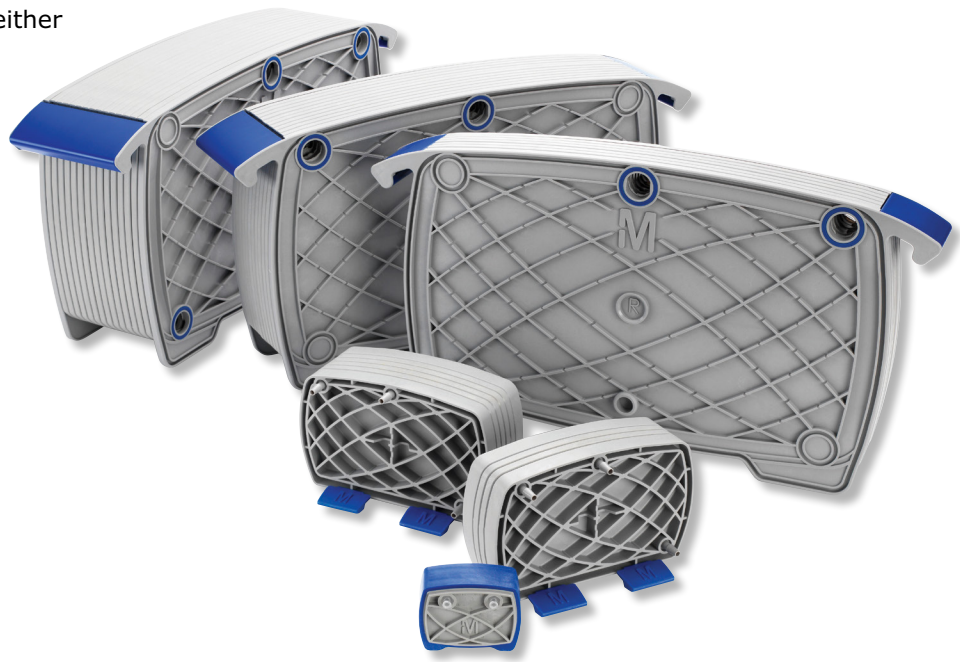


Table 1. Pmax™/Tmax™ Method for Depth Filter Data Collection Sheet

Product:		**Date:**	
Test flow rate (mL/min):		**Initial product turbidity (NTU):**	
Primary depth filter:		**Cell density (x10^6 cells/mL)**	
Catalog number:		**Cell viability (%):**	
Lot number:		**Product concentration (g/L):**	
Filtration area (cm²):			
Secondary depth filter:			
Catalogue number:			
Lot number:			
Filtration area (cm²):			

Product Filtration					
Time (min)	**Volume (mL)**	**Pressure 1 (psi or bar)**	**Pressure 2 (psi or bar)**	**Filtrate turbidity (NTU)**	**Comment**
2.5			-		
4.5			-		
6.5			-		
8.5			-		
10.5			-		
12.5			-		
14.5					
16.5			-		
18.5			-		
20.5			-		
22.5					
24.5			-		
26.5			-		
28.5			-		
30.5					
32.5			-		
34.5			-		
…					

Stop when pressure has reached 22 psi (1.5 bar) OR when the filtrate turbidity increases.

The following information should be recorded at the end of the experiment:

- Final volume (mL)
- Final pressures (psi bar)
- Filtrate pool turbidity (NTU)

Data Analysis and Sizing

Before starting experimental analysis, ensure that the units used in all calculations are as listed below:

Volume (V):	Liters (L)
Filtration area (A):	Square meters (m²)
Time (t):	Hours (h)
Pressure (P):	Pound-force per square inch (psi)

If the test stopped due to a pressure increase (22 psi/1.5 bar), sizing should be made according to Pmax™ test method (section 3.1).

If the test stopped due to a turbidity breakthrough, Tmax™ test method should be used section 3.2.

3.1 Filter sizing using Pmax™ test method

To determine filter sizing using the Pmax™ method, the data are analyzed as follows:

1. Calculate your filtrate loading (L/m²): divide your filtrate volume (L) by filter area (m²).

   For two-stage clarification, perform the calculation on the primary depth filter and apply the 2:1 ratio on the secondary depth filter (secondary depth filter should be half the area of the primary).

2. Calculate the filtrate flux in L/m²/h (LMH) by dividing the pump flowrate (L/h) by the test device area (m²).

3. Calculate the filter resistance (psi/LMH) by dividing the upstream filter pressure (psi) recorded during the trial by the filtrate flux (LMH) calculated at step 2.

4. Plot resistance (psi/LMH) against filtrate loading (L/m²) as shown in Figure 4.

5. Select the desired process endpoint pressure.

6. Select (estimate) an initial area $A_{selected}$ for full process run.

7. Calculate capacity in L/m²: Process Volume/ $A_{selected}$.

8. Calculate process flux in LMH: Process Volume/ (Process time* $A_{selected}$).

9. Calculate resulting pressure endpoint (psi): from the graph, find the resistance (psi/LMH) corresponding to the capacity (L/m²) value calculated in step 7. Pressure endpoint is equal to that resistance (psi/LMH) times process flux (LMH):

Pressure = Flux x Resistance

with

$$\text{Flux} = \frac{V_{process}}{(A_{selected} \times Time_{process})}$$

10. Compare the calculated pressure to the selected pressure at step 5.

11. If not equivalent, select new area and repeat step 6–10 until pressures are equivalent. Once the equation is resolved, $A_{selected}$ is the minimum area (A_{min}) required for the process.

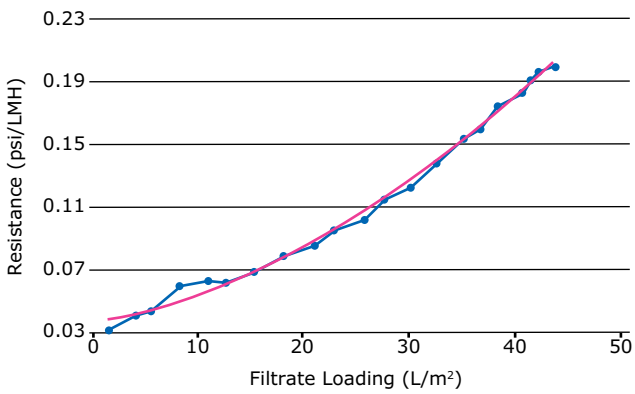


Figure 4. Depth Filter resistance over loading

3.2 Filter sizing using Tmax™ test method

To determine filtration area from a Tmax™ test, plot the filtrate turbidity (NTU) against the filtrate loading (in L/m²) as shown in Figure 5.

Based on this curve, the filter capacity (L/m²) is read directly from the turbidity shift.

Minimum filtration area A_{min} is calculated as follow:

$$A_{min} = V_{process} / C$$

A_{min} is the minimum process area calculated (m²)
$V_{process}$ is the batch volume (L)
C is the filter capacity (L/m²) read at the point of turbidity shift on the graph

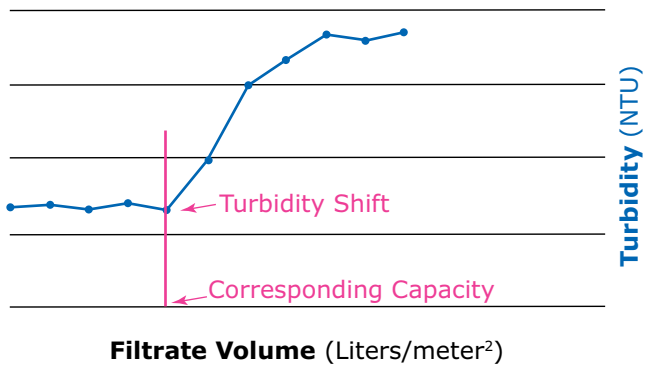


Figure 5. Turbidity vs. volume filtered

3.3 Sizing calculations

The value for A_{min} determined by one of the methods above is the minimum surface area for a process. The recommended filtration area for safe operation is based on A_{min} and a safety factor to accommodate process variability:

$$A = A_{min} * SF$$

Where:
A is the final process area (m²)
A_{min} is the minimum process area calculated (m²)
SF is the safety factor (1.3 - 1.9)

To finish, process parameters can be calculated:

Table 2. Typical range of safety factors

Application	Typical safety factor range	Initially recommended safety factor (without detailed information)
Clarification	1.3-1.9	1.5

* Further information can be found in Journal of Membrane Science 341 (2009) p268–27.

Pmax™ method sizing

Process flux is calculated:

$$Q_{process} = V_{process} / (T_{process} * A)$$

Tmax™ method sizing

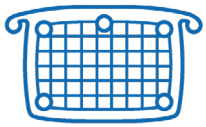
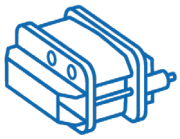
Estimated process time is calculated:

$$T_{process} = V_{process} / (Q_{process} * A)$$

Where:
$Q_{process}$ is process flux in LMH
$V_{process}$ is the batch volume (L)
$T_{process}$ is the expected process time (h)
A is the filtration area (m²), including SF.

Scale-up

The pilot trial should be a scaled version of the system filter train recommended in Pmax™/Tmax™ testing. The batch volumes, flow rates and temperatures should be as close as possible to lab-scale trials. The recommended scale-up strategy is as follows:

Lab Scale	**Pmax™/Tmax™ method** (this protocol) µPod® devices	
Pilot Scale	**Pilot trials with a pump** Process scale Pod or Lab scale Pod devices. Apply conditions defined at lab scale. Follow filtrate volume, pressure and tubidity over time.	
Manufacturing Scale	**Final process with a pump** Respect system inlet pressure and Pod differential pressure during runs	

For more information on Pmax™ method, please reference our Application Note AN1512EN00.

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