

CUNO Application Brief

Chromatography Column Protection with CUNO LifeASSURE[®] PB Membrane Filters

Introduction

Protective, or guard, filters are often used in chromatography column purification steps to protect the column media from contaminants in the various fluids that come in contact with the column. These fluids include not only the product being purified, but also the buffer solution used to elute the column, as well as rinse water and regeneration and CIP chemicals. All these fluids, if not clean, can result in poor column efficiency, channeling of the media, blockage of the inlet frit, or bacterial contamination of the column.

This Application Brief discusses an alternative chromatography column protection filter, LifeASSURE PB, that:

- 1. Provides extremely high retention of *Brevundimonas diminuta*, the test organism used to validate retention of membrane filters, and
- 2. Is compatible with CIP chemicals such as sodium hydroxide.

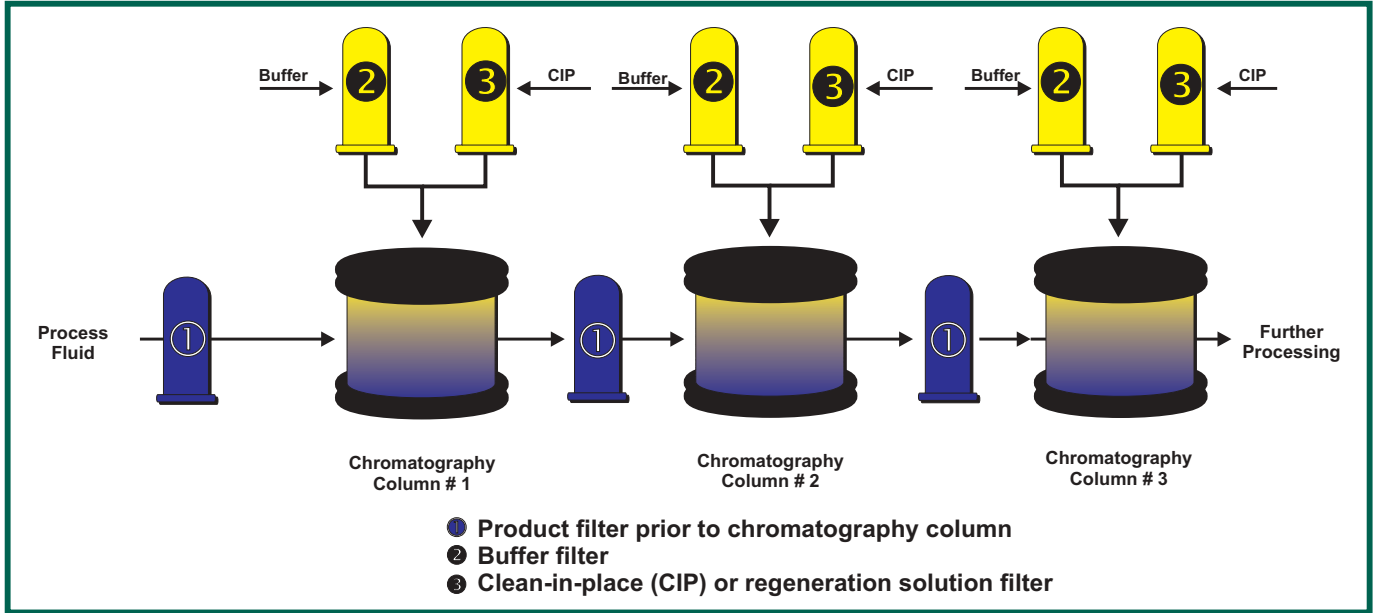
Frequently, the pharmaceutical and bioprocess industries have employed sterilizing grade 0.2 μm rated filters for chromatography column protection. Sterilizing grade filters, such as the CUNO Zetapor[®] 020SP filter, are those that meet the FDA definition and have been qualified for complete retention of a test organism, *Brevundimonas diminuta*, at a minimum concentration of 10^7 CFU/cm² of membrane surface area, as described in the American Society of Testing and Materials (ASTM#F838-83) methodology for sterilizing grade membrane filters. This style of filter was developed for use in final sterile filtration of drug products prior to packaging, where absolute sterility is mandatory.

However, their use as a protective filter in a chromatography column operation is arguably unwarranted and costly, since fluids coming into contact with the chromatography column typically have no, or very low, levels of bioburden, and the column itself is upstream of final sterilizing grade filtration located further downstream in the process. The majority of chromatography column manufacturers agree that sterilizing grade membrane filters are not required to protect the column, as long as the column is adequately protected from particulate, cell debris, and bacteria.

Additionally, many sterilizing grade membrane filters are made of polymers, namely polyvinylidene fluoride (PVDF), that are not compatible with chemicals typically used to regenerate chromatography columns, such as sodium hydroxide. This forces the operator to bypass the filter while regenerating the column, adding complexity and cost to the system.



The Process: Chromatography Column Protection



A purification process typically has between one to four different chromatography steps, each using different chromatography media and targeting a different separation goal. Each step must be protected from impurities in the process itself, impurities and bioburden in the buffer solutions, and impurities in the CIP solutions. In many cases, this process schematic may be simplified by allowing buffers and CIP solutions to share the same line. This reduces the number of lines entering the column, but care must be taken to ensure that the filter selected is compatible with both the buffer and the CIP solution.

The Problem: Chromatography Column Fouling

Chromatography columns are an expensive and necessary separation step for many bioprocess agents. As such, great care is exercised in maximizing their performance.

Typical contaminating agents that can damage column performance include the following:

- Lipids, DNA, and viruses from fermentation processes
- Bacteria or other bioburden in the process fluid, elution, or buffer solutions
- Particles or other undissolved materials in product, buffer or CIP solutions
- Endotoxins in rinsing water

These impurities can result in poor column efficiency, channeling of the media, blockage of the inlet frit, or bacterial contamination of the column. A worst case scenario could involve reprocessing of a batch, resulting in inordinate process costs and potential compromise of the validation process in the eyes of regulatory agencies.

The CUNO Solution

CUNO LifeASSURE PB filters provide exceptional bioburden and particulate control as well as high resistance to sodium hydroxide regeneration solutions commonly used to regenerate chromatography columns.

The LifeASSURE PB filter incorporates CUNO's advanced FlexN™ Nylon 6,6 membrane technology (US and foreign patents pending). As the SEM photo of the LifeASSURE PB membrane cross section below demonstrates (Figure 1.), the membrane is constructed with a single layer of membrane consisting of an "open" zone on the upstream side of the membrane and a "tighter" zone on the downstream side. In ef-

fect, the upstream zone acts as a prefilter, capturing larger particles and bacteria, while the tighter downstream zone provides retention of smaller particles and bacteria. This multi-zone structure results in greater contaminant capacity, while maintaining fast flow rates. The combination provides the end-user with the security and reliability of consistently high bacteria removal along with the enhanced economics of longer lasting, faster flowing filter assemblies.

Two particular areas were investigated in relation to the suitability of LifeASSURE PB filters for protecting chromatography columns. First, typical microorganism reduction data were generated. Additionally, experiments were conducted to determine the effect of exposure to sodium hydroxide on the bacteria retentive qualities of the filter.

Microorganism Retention

To evaluate the efficiency of submicron filters, bacterial retention studies are often performed. Bacteria retention studies are not only extremely sensitive indicators of efficiency, they also address the primary need in biopharmaceutical filtration applications, namely, bioburden control. Bacteria retention studies were conducted using the test system shown in Figure 2. Bacteria retention testing of submicron filters has been well described. Essentially, a monodispersed solution of a known bacteria is used to challenge the test filter. The test filter filtrate is monitored by passage through an analysis membrane filter disc which is subsequently incubated for enumeration of bacterial colonies. A comparison of the number of influent bacteria to the number of bacteria in the filtrate is made. The comparison is often expressed logarithmically according to the formula: Log Reduction Value (LRV) = \log_{10} (number of bacteria entering the filter/number of bacteria exiting the filter).

In tests conducted with *Brevundimonas diminuta* (considered one of the smallest bacteria), LifeASSURE PB PLA020 (0.2 µm) and PLA045 (0.45 µm) grade filters exhibited average log reduction values depicted in Table 1.

For further details regarding this test, please refer to the CUNO Regulatory Support File for LifeASSURE PB filters, literature number LITDRSFLA.

To demonstrate this high level of microorganism reduction, consider the following example.

A buffer used to rinse a chromatography column has a specification of less than 10 CFU/ml, or 10,000 CFU/liter. The typical volume used of this buffer is 10,000 liters. Therefore, in a worst case scenario, the maximum bioburden presented to a filter would be 1×10^8 CFU (10,000 CFU/liter x 10,000 liters). If a single 10" long LifeASSURE PB filter was employed to filter this buffer, with a surface area of approxi-

Figure 1 - LifeASSURE PB Membrane Cross-Section

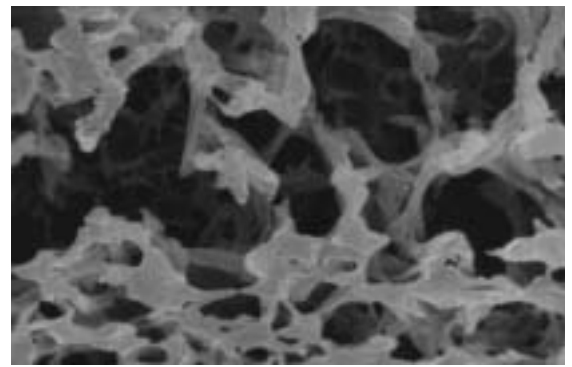


Figure 2 - Bacteria Retention Test Stand

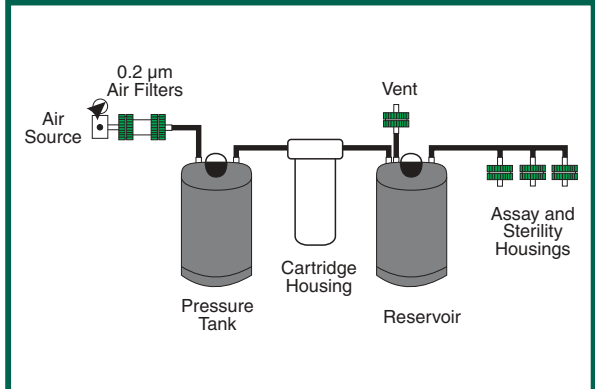


Table 1 - Log Reduction Values

Filter	LRV
LifeASSURE PB, PLA020	7.3
LifeASSURE PB, PLA045	3.5

mately 10,000 cm², then the “challenge” to the filter would be the total bioburden divided by the surface area of the filter (1 x 10⁸ CFU/10,000 cm²) or 1 x 10⁴ CFU/cm².

Since LifeASSURE PB PLA020 filters typically provide an LRV in excess of 7, the following equation can be employed to determine the probability of bacteria passage into the filtrate: challenge concentration/typical reduction, or (1 x 10⁴ CFU/cm² / 1 x 10⁷ CFU/cm²) with a result of 0.001 CFU/cm². In this worst case scenario, this is the number of organisms expected, statistically, to pass a LifeASSURE PB PLA020 filter in 10,000 liters of buffer.

Even though this probability is remote as depicted, the assumptions used in the above illustration are “worst case” making the probability of bacteria passage even less probable. For instance, the test organism, *B. diminuta*, at 0.3 µm-0.4 µm x 0.6 µm -1.0 µm, is smaller than almost all bacteria and therefore more difficult to retain than those found in actual buffer solutions. Additionally, the bacterial load of the buffer is relatively high and not likely, in operation, to contain such a high level of bacteria. In fact, many solutions, especially regeneration or CIP solutions, are toxic to bacteria and are essentially sterile in the concentrations employed.

The Effect of Sodium Hydroxide Exposure on Microorganism Retention

Sodium hydroxide is a common regeneration and sanitation agent for chromatography columns. As noted earlier, many filters, particularly those made with hydrophilic polyvinylidene fluoride (PVDF) such as the Millipore Durapore*, are not compatible with sodium hydroxide and must be bypassed when the column is regenerated and sanitized. This adds additional complexity and cost to the system.

In the following study, the effect of sodium hydroxide on the microorganism retentive ability of LifeASSURE PB filters was examined.

LifeASSURE PB cartridges were soaked in a 2.5% solution of NaOH at 60°C in 5 hour test cycles. At the end of a test cycle, each cartridge was removed from the test solution and flushed with cold water for five (5) minutes at a flow rate of 1 - 1.5 gpm. At the end of the cold water flush, each cartridge was integrity tested and then subjected to the next test cycle. The integrity test employed, the Forward Flow Integrity Test or FFIT, is a non-destructive method to determine integrity of the filter.

As the data in Table 2 show, the LifeASSURE PB filters remained integral through 50 hours of cyclical exposure.

Table 2 - Integrity Test Results LifeASSURE PB Compatibility with Hot Caustic												
Cartridge	Sample Identification	FFIT Values (cc/min)										
		Initial	5 Hours	10 Hours	15 Hours	20 Hours	25 Hours	30 Hours	35 Hours	40 Hours	45 Hours	50 Hours
PLA020	98G111-03-0090	1.8	1.8	1.7	2.1	1.5	1.8	1.8	1.9	2.4	2.3	1.9
PLA045	98H028-07-0089	1.1	1.9	1.1	1.5	0.9	1.2	1.5	1.8	1.7	1.5	1.7
PLA065	98F090-04-0142	1.0	1.2	1.3	1.4	0.9	1.5	1.4	1.6	1.5	0.8	1.3
PLA080	98G103-02-0307	0.5	0.8	0.9	1.0	0.7	1.2	0.8	1.2	0.9	0.3	1.0

Additionally, LifeASSURE PB filter cartridges were soaked for three days (72 hours) in a 1.5 M solution of NaOH at ambient temperature. After soaking, each cartridge was flushed with 30 gallons of 18 MΩ water at 3 GPM. After flushing, each cartridge was subjected to a FFIT.

*Durapore is a trademark of Millipore Corporation

As the data in Table 3 show, the LifeASSURE PB filters remained integral through 72 hours of constant exposure.

Table 3 - Integrity Test Results LifeASSURE PB 72 Hour Compatibility with Ambient Temperature Caustic				
Cartridge	Sample Identification	Initial FFIT (cc/min)	Pre-Challenge FFIT (cc/min)	Post-Challenge FFIT (cc/min)
PLA020	00D033-08-0570	1.8	3.2	5.0
	00D033-08-0559	1.8	2.8	6.6
	00D033-08-0562	1.8	2.6	2.8

Lastly, each cartridge from Table 3 was challenged with 10 liters of a *Brevundimonas diminuta* culture (minimum challenge concentration of 1.0×10^7 CFU/ml) at a flow rate of 5.0 GPM.

Table 4 - <i>Brevundimonas diminuta</i> Retention Results					
Cartridge	Sample Identification	Flow Rate (GPM)	Total Number of Organisms in Challenge (CFU)	Total Number of Organisms per cm^2 (CFU/ cm^2)	LRV
PLA020	00D033-08-0570*	5.8	3.0×10^{11}	2.8×10^7	7.2
	00D033-08-0559	5.8	2.9×10^{11}	2.7×10^7	7.4
	00D033-08-0562	4.1	2.2×10^{11}	2.1×10^7	7.5

* = Control

The data in Tables 2, 3, and 4 demonstrate that the LifeASSURE PB filter retains its high level of microorganism retention even after exposure to sodium hydroxide.

Flow Rates and System Sizing Comparisons

LifeASSURE PB filters, while providing consistent log reduction values of 7 at concentrations far and above those seen in actual process conditions, typically cost 20-50% less than comparable “validated” sterilizing grade filters on a per unit basis. Furthermore, LifeASSURE PB filters provide a superior flux rate (flow per unit area), often twice as fast as competitive filters, as seen in the Table 4.

Table 5 - Flux Rate (flow per unit area) Comparison		
Filter	Part Number	Published Flow Rate @ 1 psid
CUNO LifeASSURE PB	PLA020B01BA	2 GPM
Millipore Durapore	CVGL01TP3	1 GPM*

*Flow rates are from published product literature

When constructing a filter system, the higher flux rate of the LifeASSURE PB filter allows an operator to specify a smaller filter housing and fewer filters to provide a given flow rate compared to other, lower flux rate filters, thereby saving additional hardware and operational costs. As a means of demonstrating the cost savings, consider a system design requiring a filter to protect a chromatography column with a maximum flow rate of 6 GPM at a 1 psid clean pressure drop. This system would require only a single 30" CUNO LifeASSURE PB filter.

However, if the system used Millipore Durapore filters, it would require two 30" filters (or more commonly, three 20" filters) to provide the same flow rate at 1 psid. The economic impact of this would include the following costs: a larger filter housing, more filter cartridges, costlier filter cartridge disposal, greater exposure of filter materials to product and buffers, and greater CIP duration, fluids, and volumes.

Chromatography Protection - Special Circumstances

Under some processing circumstances, the user may wish to select a filter specially designed to address a particular concern facing the chromatography column. The following CUNO filters can address these specific needs.

Lipid Removal

The initial stage of chromatography column purification can be fouled by lipids released in the fermentation broth. CUNO Zeta Plus® Delipid series depth filters were developed to protect process equipment from lipids. Zeta Plus depth filters have long been employed by the Bioprocess industry in post-fermentation clarification and contain a matrix of inert cellulose and filter aids bound with an adsorptive resin. The Delipid series also contains a material with strong affinity to lipids, allowing for adsorption during the filtration run. Filter formats range from small scale capsules (Figure 3) to full production size cartridges (Figure 4) allowing for easy scale-up from bench-top to full production. Refer to CUNO literature LITZPDE1 for additional information.

Endotoxin Removal

For extra protection and to reduce endotoxin entering the column, positively charge-modified filters are used (Figure 5). CUNO Zetapor® 020SP 0.2µm sterilizing grade membrane filters were designed to remove endotoxin from water. The Nylon 6,6 membrane surface is chemically modified to provide enhanced removal of negatively charged molecules such as endotoxin. Additionally, this filter is validated to provide sterile filtration. Refer to CUNO literature LITCZR020SP for additional information.

Pleated Polypropylene Filters

In instances where chemical compatibility require the use of an all-polypropylene filter, the CUNO PolyPro®XL series of filters are employed. The pleated polypropylene filter offers exceptionally high flow rate due to its special pleating construction and is available in capsules, mini cartridges and full size cartridges with retention ratings from 0.2 µm to 10 µm. Refer to CUNO literature LITCPXLEL for additional information.

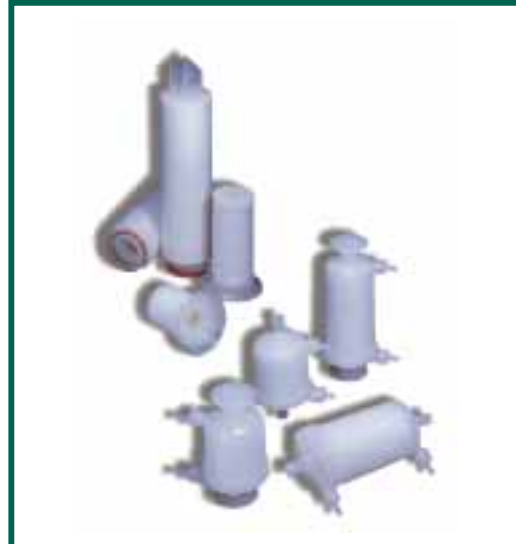
Figure 3 - Zeta Plus BioCap® Capsule Filters



Figure 4 - Zeta Plus Depth Filters and Housings



Figure 5. - Zetapor Cartridges, Mini-Cartridges, and Capsules



Conclusion and Summary

Chromatography columns should be protected against contamination from particulate, cell debris, bacteria and other materials that can foul the column, cause channeling of the media, or block the inlet frit, thereby reducing the column efficiency and reliability.

This Application Brief describes the benefits of using CUNO LifeASSURE PB filters to protect chromatography columns. LifeASSURE PB filters provide exceptionally high microorganism retention, even after exposure to sodium hydroxide, resulting reliable filtration performance. Additionally, the high flux rate provided by the unique design of LifeASSURE PB filters allows the end user to construct smaller, less costly filter systems compared to alternative filters.

References

“Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration,” ASTM Standard F838-83 (1983).

Bowmen, et al, “Microbiological Methods for Q.C. Of Membrane Filters,” J. Pharm. Sci., 56, (1967).

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“USP 23 Bacterial Endotoxin Test”(1995).

FDA “Guideline on Sterile Drug Products Produced by Aseptic Processing,” U.S. Food & Drug Administration (June 1987).

“LAL Pyrotell for the Detection and Quantification of Gram Negative Bacterial Endotoxins,” Associates of Cape Cod (ACC) (April 1990).

“FDA Guidelines on Validation of the Limulus Amebocyte Lysate Test as an End Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices,” U.S. Food & Drug Administration (1987).

Other Related Reference Literature

Reference Title/Description	CUNO Literature ID.
LifeASSURE Product Literature	LITCLAPB
LifeASSURE Regulatory Support File	LITDTRSFLA
Zeta Plus Delipid Filter Cartridges	LITZPDE1
Zetapor 020SP Filter Cartridges	LITCZR020SP
PolyPro XL Filter Cartridges	LITPXLEL

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