

Filtration of Cell Culture Growth Media and Process Buffers

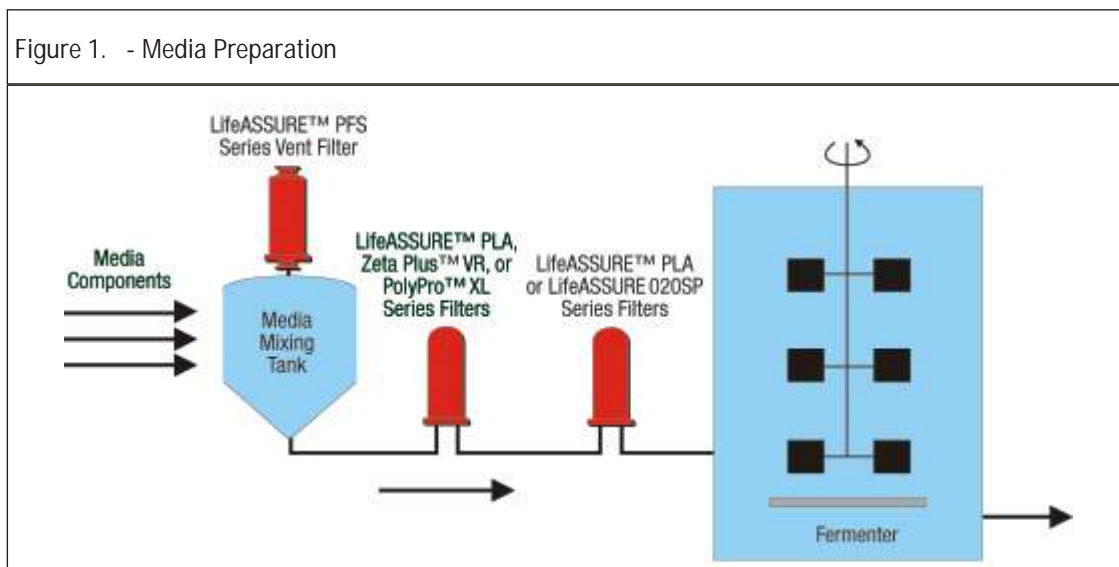
Introduction:

Media feeds and process buffers are two universal additives to biopharmaceutical processes. Media feeds are added to a fermenter to facilitate growth of mammalian, yeast, insect, and bacterial cells. Process buffers are used in conjunction with chromatography columns, for diafiltration and as a general solvent for protein solutions. Media feeds and process buffers may be formulated on site or purchased in pre-filled disposable packaging.

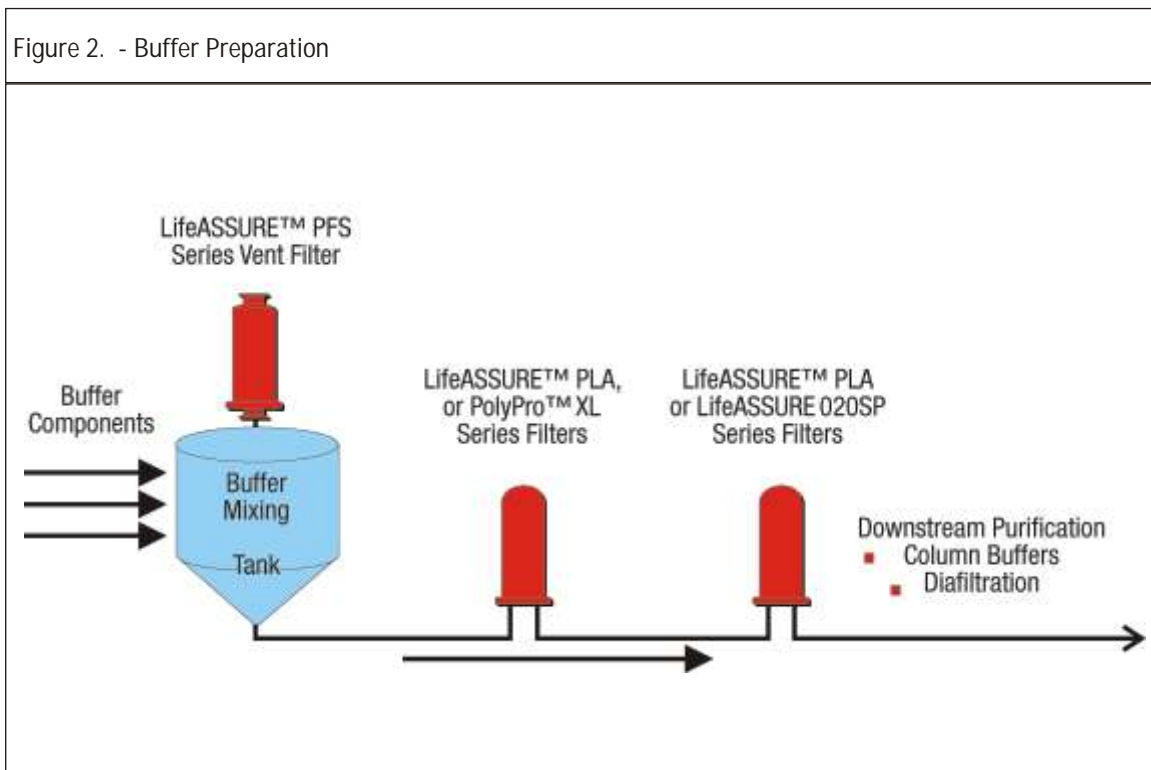
Media feeds and process buffers require filtration to protect against the introduction of bacteria, viruses, endotoxins and particulates that can contaminate and compromise processes. This Customer Application Brief presents several alternatives for filtration of media feeds and buffers.

The Process:

Media feeds consist of cell culture growth supplements added to the fermentation vessel in bulk for batch processes, or continuously in perfusion processes. Depending on the process, there may be more than one type of media feed added. Media feeds can be produced on site by mixing each of the various components in a media mix tank, followed by filtration prior to addition to the fermenter. If growth media is pumped directly from the media mix tank to the fermenter, a vent filter is required on the media mix tank to prevent ingress of bacteria. Growth media can also be purchased in pre-filled disposable packages. The location of media feed preparation in the process is shown in Figure 1.



Buffer preparation is much like media feed preparation. Buffers typically consist of water, inorganic salts and acid or base pH adjust chemicals. Each component is mixed in a buffer preparation tank and filtered prior to use. If buffers are pumped directly from the buffer mix tank to the process, a vent filter is required on the buffer mix tank to prevent ingress of bacteria. Buffers are used in downstream purification steps as shown in Figure 2.



The Problem:

Media feeds require filtration to remove bacteria and to provide additional protection from possible viral contamination. If bacteria are not removed from media feeds before they are added to the fermenter, the bacteria can enter the process and spoil the fermentation. Similarly, viral contamination of media feeds is also possible. Serum supplements may be added to growth media to support cell growth. As the serum derives from an animal source, viral contamination is always a possibility. Despite extensive steps by serum suppliers to eliminate viral contamination, the addition of a viral removal filtration step just prior to addition of serum-containing growth media to the fermentation vessel provides added protection.

Process buffers require filtration to remove bacteria and particulate matter. Bacterial contamination of process buffers not only contaminates the purification process, it also can be a source of endotoxin contamination. Endotoxins result from growth of Gram negative bacteria. If the bacteria are not removed from buffers before they have an opportunity to multiply, endotoxin contamination can result. Particulate matter must also be removed from process buffers. Particulate matter can cause fouling of chromatography columns and column frits.

The 3M Purification Inc. Solution:

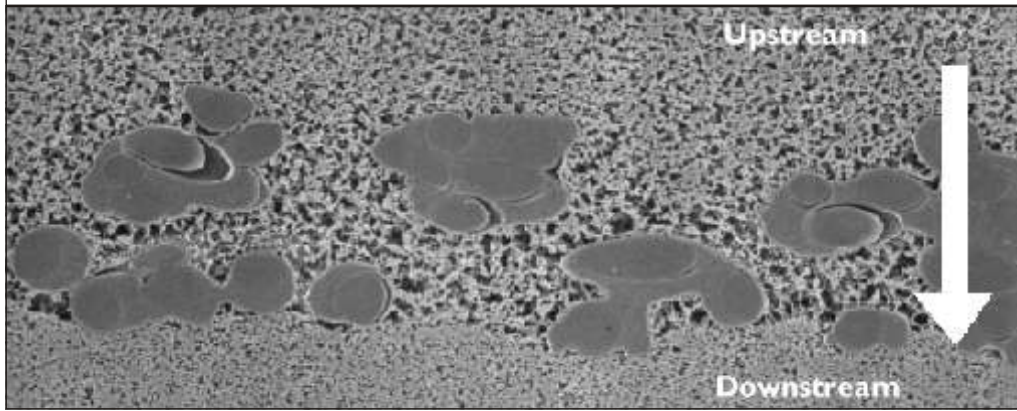
Retention of particulates and bacteria using high log reduction value (LRV) membrane filters.

LifeASSURE™ PLA series filters offer an effective means to remove bacteria and particulates from media feed and buffer solutions. In order to evaluate the bacterial retention capability of different pore size LifeASSURE PLA series filters, a series of bacterial challenge experiments were performed. The results of tests with *B. diminuta* are shown in Table 1. The results show that the average log reduction value (LRV) for *B. diminuta* with 0.20 micron rated LifeASSURE PLA series filters was 7.3, and the average LRV for 0.45 micron rated LifeASSURE PLA series filters was 3.5. Based on these log reduction values, LifeASSURE PLA series filters will provide a high level of assurance that all bacteria are removed from fluids with low (< 1CFU/1 ml) bioburden levels.

Table 1 - <i>B. diminuta</i> Retention Results					
Sample Filter	Pre-Challenge DFT	Total Challenge CFU	Challenge Level CFU/cm ²	Post-Challenge DFT	LRV
LifeASSURE PLA020, 0.20 micron, 10" filter cartridge					
98J017-04-0023	Pass	1.8 x 10 ¹¹	1.7 x10 ⁷	Pass	8.00
98J017-04-0048	Pass	2.4 x 10 ¹¹	2.2 x10 ⁷	Pass	5.93
98J017-04-0066	Pass	3.0 x 10 ¹¹	2.8 x10 ⁷	Pass	7.69
98G111-05-0052	Pass	2.3 x 10 ¹¹	2.2 x10 ⁷	Pass	7.58
98G111-03-0088	Pass	5.3 x 10 ¹¹	5.0 x10 ⁷	Pass	7.34
98G111-03-0095	Pass	1.5 x 10 ¹¹	1.4 x10 ⁷	Pass	7.29
LifeASSURE PLA045, 0.45 micron, 10" filter cartridge					
98H028-06-0023	Pass	2.7 x 10 ¹¹	2.5 x10 ⁷	Pass	3.93
98H028-07-0094	Pass	1.3 x 10 ¹¹	1.2 x10 ⁷	Pass	3.47
99E043-04-0097	Pass	1.5 x 10 ¹¹	1.4 x10 ⁷	Pass	3.22
99E043-04-0101	Pass	3.5 x 10 ¹¹	3.3 x10 ⁷	Pass	4.69
98F089-03-0165	Pass	1.7 x 10 ¹¹	1.6 x10 ⁷	Pass	2.32
98F089-03-0174	Pass	3.2 x 10 ¹¹	3.0 x10 ⁷	Pass	3.39

LifeASSURE PLA series filters also provide superior contaminant capacity for high throughput of difficult-to-filter media feeds. LifeASSURE PLA series filters are constructed of Nylon 6,6 membrane produced by a process called multi-zone microporous membrane technology. This process of membrane casting results in a multi-zone microporous structure contained within a single, continuous membrane layer. The structure of LifeASSURE PLA membrane is shown in Figure 3. The multi-zone structure contains open pores for prefiltration and tighter pores for controlled particle and bacterial retention.

Figure 3. - LifeASSURE™ PLA Series Filter Membrane Structure



In some instances it may be necessary to filter alkaline pH buffers or even caustic CIP fluids. LifeASSURE™ PLA series filters consisting of Nylon 6,6 membrane and polypropylene structural components are compatible with many of these fluids.

For low inlet bioburden applications where the LifeASSURE PLA series filter is used as a final filter, high throughput results in long filter service life. When used as a prefilter, the LifeASSURE PLA series filter offers significant life extension of final membrane filters. This is especially important for prefiltration of serum containing growth media which can cause premature plugging of 0.20 or 0.10 micron absolute rated final membrane filter cartridges.

Absolute bacteria retention and endotoxin removal

For the highest level of sterility assurance of media feeds and buffer solutions, 3M Purification's LifeASSURE™ SP 0.20 micron, sterilizing grade, absolute rated filters are recommended. LifeASSURE SP series filters are validated for absolute retention of *B. diminuta* at challenge levels in excess of 10^7 organisms per cm^2 filter area. If sterilizing grade filters are used, they should be sterilized in place (SIP) and integrity tested before use and replaced after filtration of each batch of media feed or buffer.

LifeASSURE SP series filters are also useful for removal of endotoxins from aqueous fluids. These filters are constructed with two layers of positively charged Nylon 6,6 membrane. The fixed pore structure provides absolute, size-exclusion based retention of *B. diminuta*, while the positive surface charge provides enhanced retention of smaller, negatively charged particles. Endotoxins are negatively charged lipopolysaccharide fragments of Gram negative bacterial cell walls. These molecules can contaminate buffer solutions and unless removed, enter downstream protein purification processes. Table 2 shows the results of experiments designed to evaluate the efficiency of endotoxin removal by LifeASSURE SP series filters, LifeASSURE PLA series filters and a Millipore Durapore® filter cartridge constructed of PVDF.

Filter Type	Challenge Solution	Endotoxin Challenge Concentration (EU/ml)	Total Endotoxin Challenge (EU)	Percent Endotoxin Removal
3M Purification LifeASSURE 0.20 Micron	SWFI*	600	1.2×10^6	99.98%
	Buffer **	600	1.2×10^6	83.46%
3M Purification LifeASSURE 0.20 Micron	SWFI*	600	1.2×10^6	100.0 %
	Buffer **	900	1.8×10^6	98.73%
3M Purification LifeASSURE PLA 0.20 Micron	SWFI*	600	1.2×10^6	26.67%
	Buffer **	512	1.0×10^6	2.87%
Millipore Durapore® 0.22 Micron	SWFI*	600	1.2×10^6	0.00%
	Buffer **	600	1.2×10^6	0.00%

*SWFI: sterile water for injection ** Buffer: 0.1 M Potassium phosphate, pH 7.0

The results show the highest level of endotoxin removal by the LifeASSURE™ SP series filter followed by the LifeASSURE™ PLA series filter and Millipore filter cartridge, respectively. Because endotoxins are significantly smaller than the pore size of LifeASSURE SP series filters, the high level of observed endotoxin removal is attributed to the filter's positive charge. It is noteworthy that the LifeASSURE PLA series filters and Millipore filter cartridges, which do not incorporate positive charge modification, show reduced levels of endotoxin removal despite having similar pore size as the LifeASSURE SP series filter. The enhanced endotoxin removal by positive charge is based on electrokinetic interaction. As can be seen in Table 2, this removal effect is reduced in solutions such as buffers which contain ionic species. These ionic species compete for positive filter adsorption sites and reduce electrostatic attraction of negatively charged endotoxin particles.



Virus removal from serum based media feeds with Zeta Plus™ VR Series Filters

The growth of mammalian cell cultures may require the addition of animal serum to nutrient feed streams. Typically, these growth medium supplements are well controlled by the supplier to be free of viral contaminants, however the need for additional protection is always desirable. Zeta Plus™ VR Series filters offer an easy way to provide additional viral clearance assurance with a disposable, single use sterilizable filter cartridge.

Zeta Plus VR Series filters are a family of depth filtration cartridges and capsules, constructed of cellulosic media which retains viruses by ion exchange adsorption. They are composed of high area process filter aids embedded in a cellulose fiber depth filter matrix. During the manufacturing process, a cationic charge modifier is chemically bound to the matrix component, forming a permanent, interconnected, rigid depth filter with positively charged electrokinetic capture sites. The resulting porous depth filter structure is a tortuous network of adsorptive flow channels capable of retaining contaminating viruses by anion exchange adsorption.

At the IBC Second International Symposium on Viral Clearance in 1998, Dr. Revie presented the data in Table 3 regarding the log reduction value (LRV) of several mammalian viruses from various Cohn process blood plasma fractionation steps.

Table 3. Viral Clearance from a Blood Based Protein Solution.					
Process Step	Cumulative Virus Titer Reduction (Log10)				
	BVD	EMC	HIV	PPV	PRV
Solvent Detergent	> 4.3	—	> 5.3	—	> 7.3
Supernatant III	1.4	4.3	6.1	4.7	3.8
Zeta Plus™ VR03 Grade Depth Filter	4.8	4.5	4.7	3.7	5.4
Total Cumulative Reduction	>10.5	8.8	> 16.1	8.4	> 16.6

The results in Table 3 show viral clearance for a number of process steps. In all cases, the viral log clearance observed with the Zeta Plus™VR Series Depth filter is significant. These results demonstrate the viral clearance effectiveness of Zeta PlusVR Series filters from blood based protein solutions. For serum based media feed filtration, Zeta Plus VR Series filters can be used upstream of LifeASSURE PLA series filter or LifeASSURE™ SP final filters.

Filtration of extreme pH and solvent buffer solutions with 3M Purification Inc. PolyPro™ XL series filters.

Some buffer solutions may have properties that are not compatible with organic membrane polymers. Examples are solutions with extreme pH (<3, >10) or certain solvents. In many cases these buffer solutions may be compatible with all polypropylene PolyPro™ XL series filters. These filters offer particulate removal to 0.20 micron and are constructed with a gradient density polypropylene configuration. The gradient density structure contains larger pores at the upstream filter side with progressively smaller pores toward the downstream filter side. This structure maximizes contaminant capacity ensuring long filter life. PolyPro XL polypropylene filter medium is pleated using method called Advanced Pleat Technology. This method of construction ensures that the pleating process and media support materials work together to provide longer service life than standard radial pleat construction.

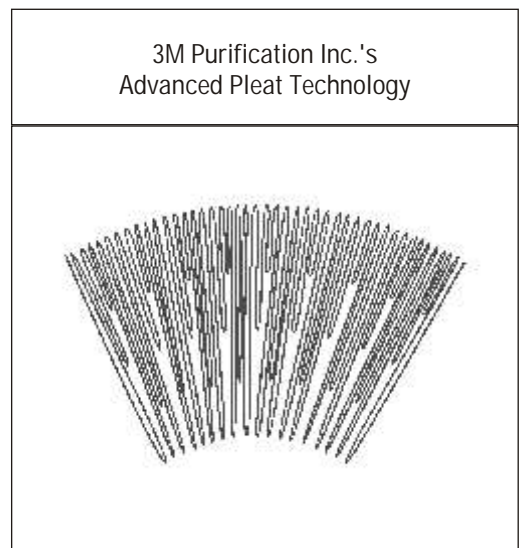
Conclusion and Summary:

This Customer Application Brief has presented a number of options for filtration of media and buffer feed solutions. In selecting the best filter or combination of filters, it is necessary to understand and define the objectives of filtration.

LifeASSURE™ PLA series filters can be used to provide reliable bacteria removal from buffer and media feed solutions. LifeASSURE PLA series filters are also an excellent choice to provide prefiltration to sterilizing grade 0.20 and 0.10 micron absolute-rated series filters, and LifeASSURE PLA series filters are compatible with alkaline pH buffers and caustic CIP fluids.

Where absolute sterilizing-grade filters are required, 3M Purification Inc.'s LifeASSURE™ SP series filters are recommended. These positively charged Nylon 6,6 filters are validated for absolute *B. diminuta* retention and the positive charge provides enhanced removal of endotoxins. LifeASSURE SP series filters should be used where filters will be replaced after each use and where the filter will be sterilized in place and integrity tested prior to use.

In some instances serum supplements to mammalian cell culture growth medium may be required, and in these cases Zeta Plus VR Series filters provide additional assurance of viral clearance. Zeta Plus VR Series filters retain viruses by an adsorptive mechanism resulting



from a cationic resin system used in the manufacturing process that provides a positive charge to the Zeta Plus VR filter medium. Zeta Plus VR Series filters may be used upstream of either LifeASSURE PLA 0.20 or 0.45 series filters, or LifeASSURE SP series filters.

PolyPro XL series filters contain all polypropylene medium that offers compatibility with buffer solutions of extreme pH and many solvents. Their gradient density polypropylene medium coupled with high surface area resulting from Advanced Pleat Technology, provides long service life and high flow rates.

Related Reference Literature

Reference Description	Literature Number
LifeASSURE™ PLA Series Filter, Product Literature	70-0201-8713-7
LifeASSURE™ PLA Series Filter, Regulatory Support File	70-0201-8821-8
LifeASSURE™ PLA Series Filter Technical Paper	70-0201-8812-7
Zeta Plus™ VR Series Filter, Product Literature	70-0201-8875-4
Zeta Plus™ VR Series Filter, Regulatory Support File	70-0201-8828-3
LifeASSURE™ 020SP Series Filter, Product Literature	70-0201-8738-4
LifeASSURE™ 020SP Series Filter, Validation Guide	70-0201-8844-0
Mini Cartridge Housing	70-0201-8886-1
Sanitary Housing, ZVS, ZMS	70-0201-8883-8
Sanitary Housing, ZWB	70-0201-8884-6
LifeASSURE™ PLA Series Filter, Installation Integrity Testing	70-0202-2027-6

Scientific Applications Support Services

The cornerstone of 3M Purification Inc.'s philosophy is service to customers, not only in product quality and prompt service, but also in problem solving, application support and in the sharing of scientific information. 3M Purification Inc.'s **Scientific Applications Support Services (SASS)** group is a market-oriented group of scientists and engineers who work closely with customers to solve difficult separation problems and aid in the selection of the most effective and economical filtration systems. 3M Purification offers specialized support to the pharmaceutical and biotechnology industry through our **Validation Support Services Program**. SASS routinely provides end-users with:

- Validation And Regulatory Support
- Extractable And Compatibility Analysis
- Filter System Optimization Studies
- CUNOCheck™ 2 Integrity Tester Validation.

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