Filtration Processes Applied in Therapeutic Monoclonal Antibody Production

Introduction

Monoclonal antibodies were among the first biotechnology produced drugs approved by the FDA and are used to treat specific diseases, as ligands in purification schemes and for use as diagnostic reagents. The primary method of monoclonal antibody production involves using murine systems to produce antibodies to specific (human) antigens. The antigens can be nucleic acid or protein molecules associated with a disease state and the antibodies directed against these antigens are exquisitely specific and identical in structure and function. In order to produce large quantities of monoclonal antibodies, the cells or genes producing the antibodies are fused with cells, typically mammalian, able to be continuously grown in suspension cell culture. The resulting cells are called hybridomas. Many monoclonal antibody producing cell culture systems transport the expressed monoclonal antibodies into the cell culture medium. The purification process begins by separating the monoclonal antibody proteins from the cell mass followed by multiple chromatographic and filtration unit operations.

This 3M Purification Inc. Application Brief presents experience gained from many monoclonal antibody purification systems using 3M Purification Inc. filtration products at the various filtration purification stages.

The Process

The process to purify monoclonal antibodies from fermentation through final filling is illustrated in Figure 1. 3M Purification Inc. filtration applications described include: media preparation, cell separation, protein concentrate clarification, chromatography column protection and prefiltration prior to final sterilizing filtration.

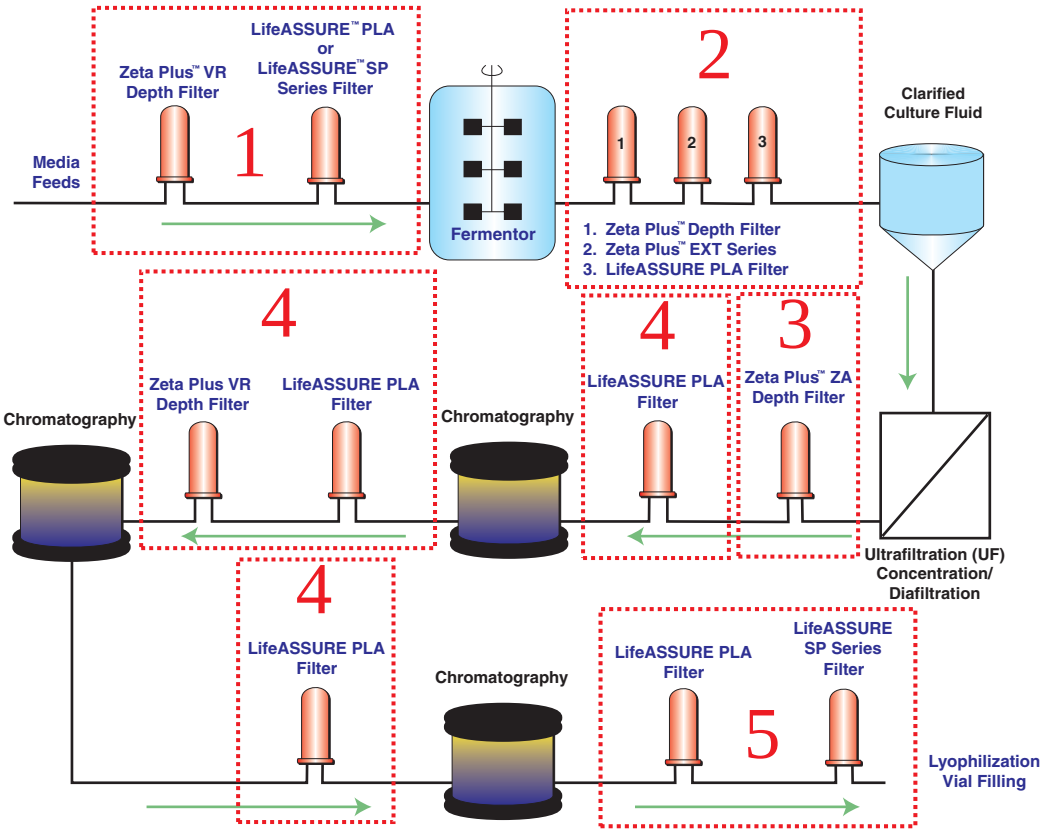

Figure 1 — Typical Manufacturing Process for Ancillary Chemicals

The Problem

Filtration issues associated with the processing of monoclonal antibody production can be segmented into the following categories. For filtration location in the process, refer to Figure 1.

1. Media Filtration

Filtration is a significant part of the overall purification process of monoclonal antibodies. Large scale monoclonal antibody production processes employ suspension cell culture to grow hybridoma cells which typically secrete monoclonal antibodies into the fermenter fluid. Prior to fermentation however, growth supplements are added to the fermenter vessel to support cell growth. These supplements, called media feeds, can contain contaminants such as virus and bacteria, which can contaminate the fermentation process if not removed. Virus contamination can result from serum containing additives used to supply necessary cell growth factors. Because of the concern with viral contamination, many processes now use serum free media feeds. Even though the possibility of viral contamination may be reduced, filtration is still required to protect against bacterial contamination.

2. Cell Separation

Once media feeds have been added to the fermenter, the process continues until cell growth and antibody production reach optimal levels. At this time the purification process begins with harvest of the fermentation vessel, requiring separation of cell mass from monoclonal antibodies secreted into the culture fluid. Problems associated with the cell separation stage include, yield loss of monoclonal antibodies, cell rupture causing release of proteases, difficulty of filtration based on cell viability and cell number and insufficient removal of cell debris which can compromise downstream purification unit operations.

3. Clarification of Protein Concentrate

Once the cell debris has been sufficiently removed from the culture fluid, purification of monoclonal antibodies continues by concentrating the harvest fluid to a more manageable volume for chromatographic purification. During the concentration step, precipitates can form which must be removed by filtration. If the concentrated solution is not adequately clarified, plugging of downstream chromatography columns can result.

4. Chromatography Column Protection

In addition to removing insoluble debris from protein solutions applied to chromatography columns, many elution buffers and regeneration chemicals applied to columns must also be filtered. One of the more common chemicals used to regenerate and sanitize columns is caustic-sodium hydroxide ranging from 0.25 to 1 molar. Caustic solutions contain particulate material that must be removed; however, many filtration media may not be compatible with caustic solutions.

5. Final Fill Prefiltration

The final step in monoclonal antibody production involves final filling of purified product. Where the final dosage form is liquid, the purified solution must be filtered through a 0.2 micron absolute rated sterilizing filter. In order to obtain maximum performance of the final sterilizing filter, bioburden exposed to the filter and any particulate matter, which can cause shortened life of the final filter, must be removed.

The 3M Purification Inc. Solution

1. Media Filtration

Numerous reagents including water, growth factors, carbon sources, pH adjusts, etc. are added to the fermentation vessel to support cell growth. These reagents may be added in bulk at the beginning of the fermentation or perfused continuously. A complete discussion of filtration applied in media preparation is available in 3M Purification Inc. Application Brief LITCABLA3. The goal of media filtration is to prevent unwanted contaminants such as bacteria and viruses from entering the fermentation vessel. Three 3M Purification Inc. products, Zeta Plus™ VR Virus Reduction Filters, LifeASSURE™ PLA filters and LifeASSURE™ SP Series

grade filters meet these requirements. Zeta Plus™ VR Series filters are recommended where serum containing growth supplements are added to media formulations. Figure 2 shows viral reduction results obtained with Zeta Plus VR Series filters for a panel of viruses spiked into a plasma fractionation process step (Cohn Fr SIII).

Table 1 — Viral Clearance from Plasma Fraction S III

Process Step	Cumulative Virus Titer Reduction (Log_{10})				
	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 VR 03 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 of viral reduction using Zeta Plus VR Series filters supports their use in reducing viral contaminants possibly associated with serum growth supplements.

In many media formulations, serum supplements are not used due to concerns with viral contamination, however, bacteria retentive filtration is still required. 3M Purification Inc. offers two options for bacteria control. LifeASSURE™ filters offer high throughput for difficult to filter media additives and bioburden control, with a typical LRV for *B. diminuta* of 7.3. LifeASSURE™ SP series grade filters offer complete *B. diminuta* retention and have a positive charge for enhanced removal of endotoxins. These attributes are summarized in Table 2.

Table 2 — LifeASSURE PLA and LifeASSURE SP Grade Filters

Filter Type	Bacteria Retention	Additional Attributes
LifeASSURE™ PLA020	Typical LRV 7.3	multi-zone microporous membrane medium for high throughput capacity
LifeASSURE SP	Sterilizing grade	Positive charge for enhanced endotoxin reduction

2. Cell Separation

One of the most difficult filtration applications is separation of cell mass following fermentation. Zeta Plus depth filtration media is the separation method of choice for this application. For complete details of cell separation, please see 3M Purification Inc. Application Brief LITCABZPS1 and “Clarification of Animal Cell Culture Process Fluids Using Depth Microfiltration”, Singhvi et al, BioPharm 9, Vol 4, April 1996. The goal of the cell separation stage is to remove cell mass, allowing the monoclonal antibody protein to pass. The final filtrate resulting from depth filtration must be able to pass through a 0.2 micron rated filter prior to further downstream purification. The factors most affecting this filtration step are cell density, cell viability and flux of filtration.

Cell density for monoclonal antibody fermentation can range from 10^6 cells/ml to 10^7 cells/ml and the higher cell density results in reduced throughput per square foot of filtration media employed. The effects of cell viability and flux are shown in Figures 2 and 3.

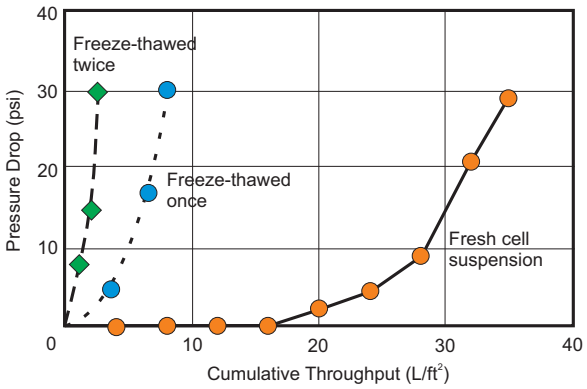

Figure 2 — Filter Throughput vs. Cell Condition

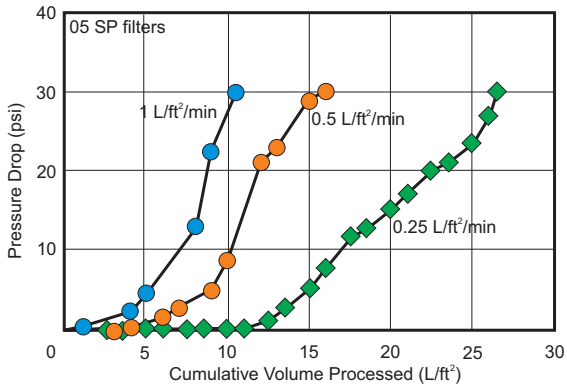

Figure 3 — Filter Throughput vs. Flux Rate

Figure 2 shows throughput results with fresh cells and cells frozen and thawed once or twice. With increasing freeze/thaw cycles, cell viability decreases as cellular debris is generated, making filtration more difficult. Figure 3 shows the effect of flux (flow per unit area) on throughput. As flux increases, throughput decreases. The optimum flux for mammalian cell culture clarification using Zeta Plus™ filters is 0.25-0.5 L/min/ft^2. Larger scale (> 1000 L) cell culture systems typically require two stages of Zeta Plus depth filtration prior to 0.2 micron filtration. Standard grade or extended throughput Zeta Plus EXT grade are recommended for each stage and typical grades and throughput volumes are summarized in Table 3.

Table 3 — Recommended Grades of Zeta Plus for Cell Separation

Cell Separation Stage	Zeta Plus Grade and Type	Recommended Flux	Typical Throughput
1	05SP, 10SP, 30LA, 10M02, 30M02	0.25-0.5 LPM/ft^2 0.25-0.5 LPM/ft^2	10-20 Liters per ft^2 15-40 Liters per ft^2
2	60SP, 60LA, 60M03	0.25-0.5 LPM/ft^2 0.25-0.5 LPM/ft^2	15-30 Liters per ft^2 25-60 Liters per ft^2

Each of the Zeta Plus filters referenced in Table 3 can be steam sterilized and is designed for single use. Because Zeta Plus filters are used once and disposed following use, opportunity for cross batch contamination is eliminated and clean-in-place (CIP) validation is substantially reduced.

3. Clarification of Protein Concentrate

Following cell separation, the clarified culture suspension is filtered through a 0.2 micron rated membrane filter and stored for downstream purification. The first downstream purification step typically involves concentration and diafiltration using an ultrafiltration membrane. The purpose of concentration is to reduce the fluid volume for easier handling and chromatographic steps. Diafiltration involves solvent exchange with the objective of completely removing cell growth media constituents and replacing them with a physiological buffer system more suited for chromatography. One of the consequences of concentration is that protein solutions often become turbid due to precipitation of denatured protein and other constituents. One or two stages of filtration may be required to clarify concentrated protein solutions and to remove particulates that can plug expensive downstream chromatography columns. Table 4 summarizes the filtration steps recommended for this application.

Table 4 — Clarification of Concentrated Protein Solutions

Filter Stage	Filter Type	Typical Throughput Volume
1	Zeta Plus 60ZA or Zeta Plus EXT 60M02	5- 15 L/ft^2
2	LifeASSURE PLA020, 0.2 micron	5- 15 L/ft^2

4. Chromatography Column Protection

Chromatography is the most commonly used unit operation in downstream purification and most purification schemes utilize three or more chromatographic steps. In all cases, chromatography media is expensive and consistent performance is dependent on maintaining free flow through the column. Failure to remove contaminants and debris prior to loading chromatography columns can cause column plugging and channeling. 0.2 micron rated membrane filters are recommended for filtration of product loaded onto columns and for filtration of elution buffers and regeneration chemicals. Product solution and most buffers are aqueous and do not typically cause compatibility problems with 0.2 micron rated membrane filters. Regeneration chemicals, however, are typically alkaline pH caustic fluids that can cause compatibility problems. 3M Purification Inc. LifeASSURE™ 0.2 micron rated filters are compatible with caustic regeneration chemicals and thus can be used to directly filter caustic applied to columns. In order to assess compatibility, LifeASSURE filters were exposed to 0.6 M NaOH at 60C for up to 50 hours. Each of the filters tested was determined to have maintained compatibility based on forward flow integrity test results.

5. Final Fill Prefiltration

Following purification of the desired monoclonal antibody, the final processing steps involve aseptic filling of vials or lyophilization. Aseptic filling requires final filtration using 0.2 micron rated sterilizing grade filters, or in some instances, 0.1 micron rated final filters. In order to ensure trouble-free sterilizing filter performance, a 0.2 micron rated prefilter is often used. LifeASSURE PLA020 filters are recommended for this application. The multi-zone microporous membrane LifeASSURE™ construction provides high flow rates, high bioburden reduction (LRV typically 7.3) and high contaminant capacity. These attributes ensure excellent protection of downstream sterilizing filters and economical system sizing.

Conclusion and Summary

This 3M Purification Inc. Application Brief has focused on filtration processes applied in purification of monoclonal antibodies. These processes include: filtration of growth media fermenter feeds, cell separation following fermentation, clarification of protein concentrates, prefiltration prior to column chromatography and prefiltration prior to sterilizing filtration. The problems associated with each filtration stage and the solution to these problems is presented. The recommendations for filtration at each stage are summarized in Table 5 and provide process engineers with a framework for filter selection.

Table 5 — Filter Recommendations

Monoclonal Antibody Purification Step	Recommended Filter
Growth Media	Zeta Plus™ VR for Viral Reduction, LifeASSURE PLA 0.2 micron, LifeASSURE SP 0.2 micron
Cell Separation	**Stage 1** — Zeta Plus 05SP, 10SP, or 30LA , or Zeta Plus EXT 10M02 or 30M02 **Stage 2 —** Zeta Plus 60SP or 60LA Zeta Plus EXT 60M02
Clarification of Protein Concentrate	**Stage 1 —** Zeta Plus 60ZA or Zeta Plus EXT 60M02 **Stage 2 —** LifeASSURE PLA 0.2 micron
Chromatography Column Protection	LifeASSURE PLA 0.2 micron
Final Fill Pre-filtration	LifeASSURE PLA 0.2 micron

Additional 3M Purification Inc. Literature

Title	Old Literature Identification	New Literature Identification
3M Purification Inc. Filter Systems for Bioprocess and Biological Separations	LITCATBP	70-0201-8680-8
Zeta Plus™ VR Series Filter Media	LITZPVR	70-0201-8875-4
Zeta Plus EXT Series Filter Cartridges	LITZPMEXT	70-0201-8862-2
LifeASSURE™ PLA Capsule and Cartridge Filters	LITCLAPB1	70-0201-8713-7
LifeASSURE SP Membrane Filters	LITCZR020SP	70-0201-8738-4
ZPB Model Zeta Plus Filter Housings	LITHSZPBC	70-0201-8762-4
ZWB Model LifeASSURE Filter Housings	LITZRH106	70-0201-8884-6
3M Purification Inc. Application Brief - Chromatography Column Protection with 3M Purification Inc. LifeASSURE PLA Membrane Filters	LITCABLA2	70-0201-8632-9
3M Purification Inc. Application Brief - Filtration of Cell Culture Growth Media and Process Buffers	LITCABLA3	70-0201-8633-7
3M Purification Inc. Application Brief - Zeta Plus Depth Filtration and Alternative Technologies for Cell Culture Clarification	LITCABZPS1	70-0201-8667-5

3M Purification Inc. Literature Descriptions

3M Purification Inc. Filter Systems for Bioprocess and Biological Separations — 3M Purification Inc. is a leader in advanced depth filter systems and membrane-based separations, offering a range of products for all stages of biopharmaceutical and biological processing from bench top to pilot-scale to manufacturing scale operations.

Zeta Plus™ VR Series Filter Media — The removal and/or inactivation of contaminating viruses from biotherapeutics is a requisite for ensuring final product safety. Zeta Plus VR Series cartridge depth filters remove significant levels of viruses from biological fluids. They provide validatable viral titer reduction, high flow rates, scalability, economy, disposability and ease-of-use in the biological manufacturing environment. The Zeta Plus VR Series includes specific filter media recommendations for virus removal from blood plasma proteins and bioprocess-derived cell culture fluids.

Zeta Plus EXT Series Filter Media — Now added to this well-established family of Zeta Plus filters is the ground-breaking Zeta Plus EXT Series. Featuring a new dual zone construction, Zeta Plus EXT Series filters significantly increase throughput per unit area of filter media, while optimizing the desired effluent clarity. Zeta Plus EXT filter media consists of two distinct layers, or “zones” of filter media with the upstream zone more open than the downstream zone. This structure enhances the contaminant holding capacity of the filter media, since larger particles are trapped in the upper zone of the filter media and smaller particles are trapped in the lower zone, reducing premature plugging and extending service life. These two layers can be selected independently of each other to optimize performance. Standard combinations determined to have the optimum through-puts are included in the ordering guide on the back, although the user can select custom arrangements if required.

LifeASSURE™ PLA Cartridge and Capsule Filters — LifeASSURE PLA filter cartridges and capsules are our latest advance in membrane filter technology. Encompassing two leading-edge processes, multi-zone microporous membrane manufacture and MaxMedia pleating construction, the LifeASSURE PLA series of filters offers unmatched protection of final membrane filters, as well as exceptionally long service life. Designed with pleated Nylon 6,6 membrane in an all-polypropylene cartridge construction, LifeASSURE PLA filters are ideally suited for a wide range of prefiltration and clarification applications in the pharmaceutical, biological, and bioprocess industries.

LifeASSURE™ SP Sterilizing Grade Cartridge and Capsule Membrane Filters — 3M Purification Inc. pioneered the development of charge modified Nylon 6,6 filters for the pharmaceutical industry. LifeASSURE sterilizing grade filters and capsules are validated for absolute bacteria retention and provide reliable sterile filtration performance. In addition to a fixed bacteria retentive pore structure, LifeASSURE membrane is charge modified to provide enhanced removal of negatively charged biological contaminants such as endotoxin, virus and nucleic acid fragments. The combination of a validated bacteria retentive membrane, together with enhanced removal of negatively charged contaminants, make LifeASSURE membrane an ideal choice for pharmaceutical and biopharmaceutical sterilizing applications.

Zeta Plus ZPC & ZPB sanitary filter housings provide the ultimate standard for totally enclosed Zeta Plus filter cartridge systems. Constructed from 400 grit, mirror finish, 316L stainless steel, Zeta Plus ZPC & ZPB housings meet the exacting sanitary quality standards of the pharmaceutical, food and beverage, fine chemical, and microelectronics industries. Both housing styles accommodate from one to four 8", 12", or 16" diameter Zeta Plus filter cartridges to offer a wide choice of filter media area and flow rates.

LifeASSURE ZWB sanitary design cartridge housings provide the ultimate for critical clarification and sterile filtration applications. Manufactured to the strictest of standards and from high quality 316L stainless steel, the ZWB mirror polished filter housings are designed to meet the exacting quality standards of the food & beverage industry. The ZWB series are multi-cartridge housings designed to accommodate three, five, seven, or twelve sanitary style filter cartridges up to 40 inches long.

Scientific Applications Support Services

The cornerstone of our 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. Our **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 Inc. 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.

For more information regarding Validation Support Services, please contact 3M Purification Inc. Technical Services or your local Distributor.

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