

# Clarification Solutions for Pre-treated Feed Streams

## Clarisolve® Depth Filters Clarisolve® mPAA Polymer pDADMAC Polymer

The current evolution to high cell density and high-product titer monoclonal antibody cell cultures is placing a larger burden on traditional downstream clarification and purification operations. To alleviate this bottleneck, various types of pre-treatment are being employed to make the clarification process more efficient.

Polycationic polymer modified poly (allyl amine) [mPAA] and poly (diallyldimethyl-ammonium chloride) [pDADMAC] are very effective flocculation polymers that can be used as a pre-treatment solution when used in combination with our Clarisolve® depth filters. The Clarisolve® depth filter is a primary clarification tool with a gradient density structure specifically designed to the particle size distributions of pre-treated feed streams. With improved volumetric capacity and reduced turbidity over currently available depth filters, the clarification step of pre-treated feeds can be processed in a significantly reduced footprint.

Together, the Clarisolve® mPAA and pDADMAC polymers, and Clarisolve® depth filters provide an effective solution for harvesting high cell density cultures that can be easily incorporated into current clarification platforms.



### Benefits of This Pre-treatment Platform

- Provides an efficient way to clarify high-density pre-treated feed streams
- Enables processing of pre-treated feeds in a significantly reduced footprint with reduced flushing requirements
- Eliminates the need for centrifugation and enables implementation of a single-use clarification train
- Fully scalable from bench to process scale in a modular, self-contained pod format

# Pre-treatment Methodologies

## Effective methods to clarify high-density feed streams

Pre-treatment is accomplished either through the reduction in pH of the feed stream (acid precipitation) or through the addition of cationic polymers including Clarisolve® mPAA polymer, pDADMAC, PEI, and chitosan. These methods result in aggregation of smaller particles into larger agglomerates thus reducing small particles which are difficult to centrifuge and can plug downstream filters (**Figure 1**). Flocculation technologies can also potentially reduce soluble impurities such as DNA and HCP, reducing the burden on downstream processing.

### Polymer Product Information

Clarisolve® mPAA polymer is an innovative polymer which spontaneously flocculates upon the addition of a phosphate stimulus. The purification process is designed to ensure a highly water-soluble product which has low levels of monomer, reaction precursors and residual solvents.

pDADMAC is a water-soluble polymer widely used as a flocculant in waste water treatment. Commercially available solutions of this polymer contain residual monomer and sodium chloride in significant concentrations and may come with high bioburden. We have developed a purification method that reduces the content of low molecular weight polymer and the concentration of the monomer to less than 0.1% and eliminates sodium chloride at the same time.

For both polymers, 0.2 µm filtration step is performed to reduce the microbial count to less than 10 CFU/mL. The molecular weight distributions of both polymers are shown in **Figure 2**.

### Use of Polymers in Flocculation

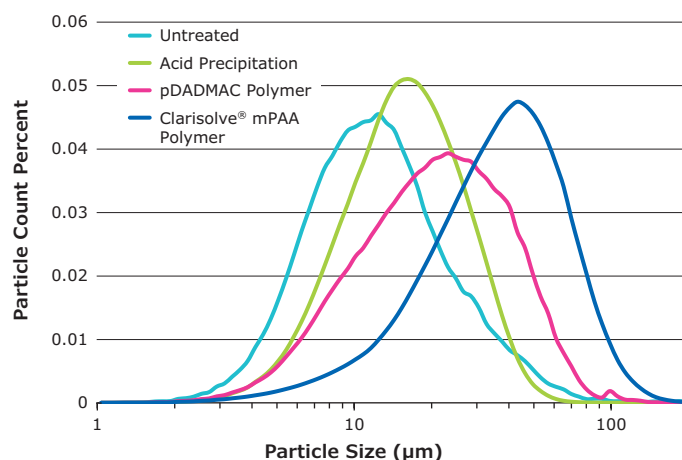
Clarisolve® mPAA polymer delivers the best flocculation results when used in a concentration of 0.1% and 0.5%. However, the stimulus-responsive nature of the polymer means that the flocculation is still effective at higher dosages. Flocculation is initiated via addition of 10–100 mM phosphate.

For pDADMAC polymer, the best flocculation results when used in a concentration between 0.01% and 0.05%.

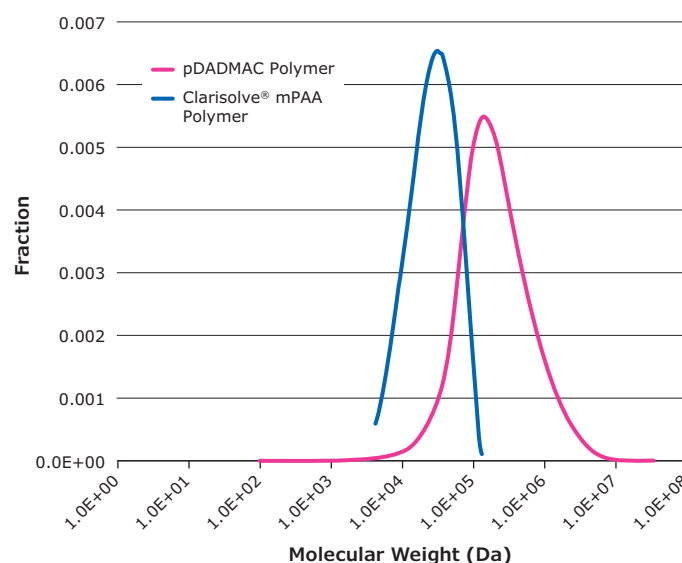
### In-Process Polymer Removal and Safety

Studies have shown that the residual concentration of the pDADMAC polymer is below 1 ppm following the downstream purification train. No cytotoxicity or acute systemic toxicity was observed at this concentration in toxicological studies, nor have irritant (intracutaneous injection) or haemolytic effects been found.

The residual concentration of the Clarisolve® mPAA polymer has been determined to be below 5 ppm, following a three-step downstream purification of a mAb feed stream. No evidence for acute systemic



**Figure 1. Particle size distribution of various pre-treatment methodologies**



**Figure 2. Molecular weight distributions of Clarisolve® mPAA and pDADMAC polymers after purification**

toxicity was observed at this concentration in a limited in vivo toxicological study. No observation of hemolytic or mutagenic effects were made during in vitro studies at this concentration.

### Quantitation of Residual Polymers

We provide a service to quantitate the residual level of Clarisolve® mPAA and pDADMAC polymers in sample matrices from a cell culture harvest and clarification before and after the purification steps.

A report is issued including a description of samples submitted, quantitation of the polymer residuals and a chromatogram overlay including the standard. Final reports are issued electronically in a PDF format and are provided in a format suitable for submission to regulatory authorities.

# Clarisolve® Depth Filters

## Successful in a variety of mAb, vaccine, and microbial lysate feed streams

With pre-treatment methodologies, the shift in particle size distribution of the flocculated feed stream may result in inefficient conventional centrifugation and depth filtration installations which may be difficult to fit into existing facilities or new facilities where footprint is limited. Clarisolve® depth filters have been optimized to match the particle size distribution of typical pre-treated feeds, resulting in improved filtration performance not seen with traditional primary clarification depth filters.

### Filtration Performance

Success with multiple monoclonal antibody (mAb) cell culture streams of varying cell densities and viabilities, including high-density cell cultures with various flocculation techniques has been demonstrated with this robust clarification technology. Clarisolve® depth filters have also been used successfully for microcarrier removal, lysate clarification, and in viral vaccine processes.

### Flushing

20MS, 40MS, and 60HX media grades can be flushed at flux rates ranging from 100 LMH to 600 LMH, depending on process and equipment capabilities. The 60HX media grade has the lowest flushing requirement among the family of Clarisolve® filter media grades.

### Device Wetting and Hold Up Volumes

All media grades exhibit excellent wetting characteristics at flush rates from 100 LMH to 600 LMH. Additionally, lower hold up volumes post blow down allow for greater product recovery over conventional depth filters.

## Selection Guide

| Pre-treatment Option                 | Clarisolve® Depth Filter |
|--------------------------------------|--------------------------|
| <b>Acid Precipitation</b>            |                          |
| Particle size of 10-20 µm            | 20MS                     |
| <b>Cationic Polymer Flocculation</b> |                          |
| Particle size of 30-40 µm            | 40MS                     |
| Particle size of 50-60 µm            | 60HX                     |

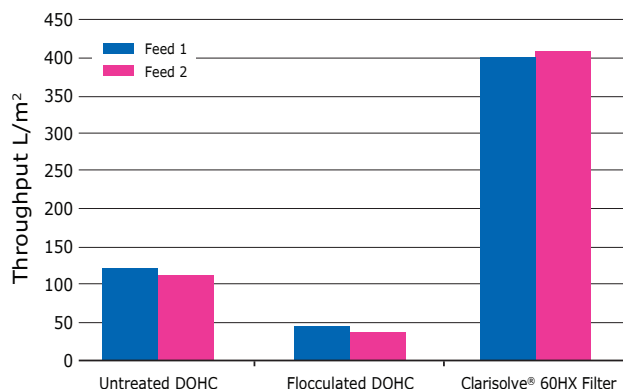


Figure 3. Filtration performance of 2 mAb feed streams highlighting the improvement in depth filter capacity with pre-treatment and Clarisolve® depth filters.

## Clarisolve® Depth Filter Format Specifications

|  | <b>µPod® Device Format</b>  | <b>Lab Scale</b>            |                      | <b>Process Scale</b>                                       |                     |                     |
|--|---|-----------------------------|----------------------|--|---------------------|---------------------|
| <b>Surface Area</b>                            | 23 cm <sup>2</sup>  | 0.014 m <sup>2</sup>        | 0.027 m <sup>2</sup> | 0.11 m <sup>2</sup>  | 0.33 m <sup>2</sup> | 0.55 m <sup>2</sup> |
| <b>Materials of Construction</b>               |   |                             |                      |  |                     |                     |
| Filter Media                                   | Polypropylene and cellulosic media with an inorganic filter aid (60HX contains polypropylene media only)  |                             |                      |  |                     |                     |
| Housing  | Glass Filled Polypropylene  |                             |                      |  |                     |                     |
| Adapters                                       | N/A   | N/A                         |                      | Glass Filled Polypropylene                                 |                     |                     |
| Gaskets and Plugs                              | N/A   | N/A                         |                      | Thermo plastic elastomer (TPE)                             |                     |                     |
| <b>Inlet, Vent and Outlet Connections</b>      | Female Luer   | 1/4" hose Barb              |                      | Flat Seal  |                     |                     |
| <b>Pod Dimensions</b>                          |   |                             |                      |  |                     |                     |
| Length:  | 3.57 in (9.07 cm)   | 8.5 in (22 cm)              |                      | 24.2" (62 cm)  |                     |                     |
| Height:  | 2.75 in (6.99 cm)   | 5.3 in (14 cm)              |                      | 12.5" (32 cm)  |                     |                     |
| Thickness (20MS and 40MS):                     | 1.96 in (4.98 cm)   | 2.9 in (7.40 cm)            | 3.7 in (9.40 cm)     | 2.0 in (5.08 cm)   | 4.4 in (11.18 cm)   | 6.8 in (17.27 cm)   |
| Thickness (60HX):                              | 1.81 in. (4.60 cm)  |                             |                      |  |                     |                     |
| <b>Maximum Operating Pressure</b>              | 50 psig (3.5 bar) @ ≤ 40 °C   | 30 psig (2.1 bar) @ ≤ 37 °C |                      | 50 psig (3.5 bar) @ ≤ 25 °C<br>15 psig (1.0 bar) @ ≤ 80 °C |                     |                     |
| <b>Maximum Differential Pressure</b>           |   |                             |                      |  |                     |                     |
| Forward:                                       | 30 psid (2.1 bar) @ ≤ 40 °C   | 30 psid (2.1 bar) @ ≤ 37 °C |                      | 30 psid (2.1 bar) @ ≤ 25 °C                                |                     |                     |
| Reverse:                                       | 15 psid (1.0 bar) @ ≤ 40 °C   | 30 psid (2.1 bar) @ ≤ 37 °C |                      | 30 psid (2.1 bar) @ ≤ 25 °C                                |                     |                     |
| <b>Sterilization</b>                           | 2 autoclave cycles of 60 minutes at 123 °C  |                             |                      | 1 autoclave cycle of 60 minutes at 123 °C                  |                     |                     |
| <b>Indirect Food Additive</b>                  | All component materials meet the FDA indirect food requirements cited in 21 CFR 177-182   |                             |                      |  |                     |                     |
| <b>Toxicity</b>                                | All component materials comply with the requirements of USP <88> biological reactivity tests for class VI plastics  |                             |                      |  |                     |                     |
| <b>USP Bacterial Endotoxins (pyrogenicity)</b> | An aqueous extraction contained less than 0.25 EU/mL as determined using the Limulus Amebocyte Lysate (LAL) clot test technique (on filter media only).   |                             |                      |  |                     |                     |
| <b>Pressure Equipment Directive 2014/68/EU</b> | Devices and associated holders are designed and manufactured in accordance with the sound engineering practices (SEP) cited in Article 4(3) of 2014/68/EU |                             |                      |  |                     |                     |

## Ordering Information

| Description   | Qty/Pk | Catalogue No.                                   |
|---|--------|---|
| <b>Clarisolve® Depth Filters in µPod® Device Format</b> |        | <b>Surface Area</b><br><b>23 cm<sup>2</sup></b> |
| 20MS Media  | 3      | CS20MS01L3                                      |
| 40MS Media  | 3      | CS40MS01L3                                      |
| 60HX Media  | 3      | CS60HX01L3                                      |
| <b>µPod® Filter Accessories</b>                         |        |   |
| µPod® Tubing Kit  | 1      | MTUBEKITL1                                      |
| Gauge 0-60 psi and Connection Fittings                  | 1      | XXPXLGAGE                                       |

| Clarisolve® Depth Filters in Lab Scale Format |   | Surface Area         |                      |
|---|---|----------------------|----------------------|
|   |   | 0.014 m <sup>2</sup> | 0.027 m <sup>2</sup> |
| 20MS Media                                    | 1 | CS20MS01H1           | CS20MS02H1           |
| 40MS Media                                    | 1 | CS40MS01H1           | CS40MS02H1           |
| 60HX Media                                    | 1 | CS60HX01H1           | CS60HX02H1           |

| Clarisolve® Depth Filters in Process Scale Format |   | Surface Area        |                     |                     |
|---|---|---------------------|---------------------|---------------------|
|   |   | 0.11 m <sup>2</sup> | 0.33 m <sup>2</sup> | 0.55 m <sup>2</sup> |
| 20MS Media  | 1 | CS20MS01F1-X        | CS20MS03F1-X        | CS20MS05F1-X        |
| 40MS Media  | 1 | CS40MS01F1-X        | CS40MS03F1-X        | CS40MS05F1-X        |
| 60HX Media  | 1 | CS60HX01F1-X        | CS60HX03F1-X        | CS60HX05F1-X        |
| <b>Disposable Adapter Kit</b>                     |   |                     |                     |                     |
| 3 through adapters, 3 blind adapters              | 1 | MP0DADAPT           |                     |                     |
| 6 through adapters, required if using MP0DDIVERTR | 1 | MP0DADPTF           |                     |                     |

**Note:** Pod filters require the use of flow adaptors which are sold separately (MP0DADAPT or MP0DADPTF). See the Millistak+® Pod disposable depth filter hardware data sheet (DS3388EN00) for information on Pod filter holders.

A retrofit kit may be required in order to accommodate the Clarisolve® depth filters in the Pod pilot and process scale holders. Please contact your local sales representative for details.

### Flocculation Polymers

|  |        |              |
|--|--------|--------------|
| pDADMAC solution 10% flocculation polymer          | 100 mL | 1.37069.0100 |
|  | 1 L    | 1.37069.1000 |
|  | 10 L   | 1.37069.9010 |
| Clarisolve® mPAA solution 10% flocculation polymer | 100 mL | 1.37099.0100 |
|  | 1 L    | 1.37099.1000 |

*Note: Clarisolve® mPAA solution should be stored in the dark at 2-8 degrees Celsius.*

### Provantage® Service

|                                  |           |
|----------------------------------|-----------|
| Quantitation of Residual pDADMAC | VSPDADMAC |
|----------------------------------|-----------|

For additional information, please visit [EMDMillipore.com/FlocculationPolymers](http://EMDMillipore.com/FlocculationPolymers)

To place an order or receive technical assistance, please visit [EMDMillipore.com/ContactPS](http://EMDMillipore.com/ContactPS)

