Cell Culture Media and Buffers in Biopharmaceutical Production

Cell culture media and process buffers are used in all biopharmaceutical operations. Culture media carries cell cultures to fermenters or bioreactors and facilitates growth of mammalian, yeast, insect, or bacterial cells. The media can be made up of numerous ingredients that are either mixed on-site or purchased pre-mixed in disposable containers.

Process buffers are used in diafiltration processes, as diluents for solutions entering chromatography columns, and as solvents or carriers for proteins. As with culture media, the buffers are often made up of multiple liquids and can be mixed on-site or pre-mixed off-site and delivered in disposable containers.

Filtration of culture media and buffers is designed to enhance the operation of fermenters, bioreactors and downstream processes.

This application guide shows the functions of filters in the preparation of media and buffers. The media and devices made by Critical Process Filtration and available to perform these functions are presented as part of the discussion. There is also a brief overview of the Critical Process Filtration technologies that may be applied in media and buffer preparation systems at the end of this guide.
Preparing Cell Culture Media

Figure 1 below is a schematic of a generic cell culture media preparation system. Once mixed, culture media is usually added to the fermentation tank or bioreactor on a batch basis. However, some operations employ continuous processing. In those cases, the cell culture media preparation system will feed the fermenter or bioreactor during the entire production run.

As the components are delivered to the mix tank, filters remove particulates and/or bacteria that may be in the components. Because the media components, by their nature, promote the growth of bacteria, assuring that unwanted bacteria are removed also assures the safe and effective operation of the fermenter or bioreactor.

If a mix tank is used, vent filters are used to maintain the quality of the solution in the tank. These filters protect the tank contents from particles and bacteria in surrounding air.

While the schematic shows one mix process, there may be multiple media prepared and fed to the fermenter or bioreactor. In such a case, there may be multiple mix tank and feed systems in place, or some media may be purchased from an outside source and delivered in rigid, reusable containers or flexible, disposable packages. In either case, the filtration needed to promote good system operation remains the same.

The locations of normal flow filters are noted in Figure 1 and their various functions are discussed in more detail later in this guide.

Buffer Preparation

Figure 2 on the following page is a schematic of a generic buffer mix system to deliver buffer to downstream processes. The buffer preparation process is much like that of cell culture media preparation. Individual components, usually water, salts and pH adjusting chemicals, are individually filtered and mixed in the mix tank. The finished solution is then delivered to the appropriate process. Buffer preparation is most frequently a batch process, but some operations may utilize continuous processing. In that case, the system must provide filtered buffer for the entire production run.
As the components are delivered to the mix tank, filters remove particulates or bacteria that may enter the mix tank. Downstream purification processes that use the buffers are sensitive to organic and particle contamination. To allow those processes to operate as intended, the finished buffer solution is filtered to remove particles and bacteria.

If a mix tank is used, the solution is protected from particle and bacterial contamination by a vent filter.

**Filter Applications**

Both Figure 1 and Figure 2 show “typical” filter applications. Additional filters may be required if either components or the mixed solutions contain materials such as gels or colloids that can cause premature filter plugging. Filters are ideal for preventing the fouling of processes such as chromatography and diafiltration, but the efficient operation of the filters is needed to make sure those processes run smoothly. Contact the Critical Process Filtration Technical Support team for assistance in finding the right filter if you are faced with an atypical contaminant in your process.

**Bioburden Reduction Filtration**

As the term implies, filters that do not remove bacteria to levels required for “sterilizing” are designated as “bioburden reduction” or “bioburden control” filters. The filter micron rating may be 0.22 μm, 0.45 μm or even 0.65 μm. As with sterilizing grade filters, the rating is a secondary consideration. Performance removing the target organism and validation of results in the application are of primary importance. The goal of bioburden reduction is to remove most organisms and promote system efficiency.

All bioburden reduction filters (Labeled “A” in Figures 1 and 2) are based on microporous membranes. Critical Process Filtration manufactures bioburden reduction filters, called “Biopharmaceutical Grade” by CPF, using asymmetric polyethersulfone (PES); high capacity PES; polyvinylidene fluoride (PVDF); high capacity PVDF; nylon 6,6; and blended cellulose membranes. Available in both cartridge and disposable capsule formats, these filters cost effectively remove most bacteria. They can be validated for performance to assure that the system will consistently deliver media or buffer that meets the requirements of your system.
Sterilizing Filtration

Typical sterilizing grade filters utilized in media and buffer preparation systems are rated to remove particles and bacteria that are 0.22 or 0.1 micron in size, but coarser filters are also available. In the past, the standard has been 0.22 micron, but a few organisms, such as *Acholeplasma laidlawii*, have been found to pass through these filters, so manufacturers have developed a finer, 0.1 micron filter to remove them.

CPF has “Pharmaceutical Grade” sterilizing cartridge and disposable capsule filters made using asymmetric PES; PVDF; nylon 6,6 or positively charged nylon 6,6 membrane. All are available with either a 0.1 μm or 0.22 μm pore size rating. Using these filters will assure that the components of your media or buffer solution are free from bacteria that could have a severe impact on your fermentation or downstream purification processes.

Tank Vent Filters

Filter D in Figures 1 and 2 are used to protect the media or buffer solutions in the tanks from bacterial and particulate contamination. As a tank is filled, the air inside must be allowed to escape. However, as the tank is emptied, air (or a process gas such as nitrogen) must be allowed to enter the tank to replace lost liquid volume. The air or gas entering the tank will have particle and bacterial content that is removed to assure the quality of the solution.

It is critical that the vent filter membrane remain dry so that air can pass freely through the filter. To assure that dryness, filters are made using hydrophobic membranes, usually PTFE or polypropylene. CPF’s PTM sterilizing grade PTFE membrane or PPM sterilizing grade polypropylene membrane cartridges or capsule filters are most often used.

If a tank is kept at an elevated temperature, then PTM/HT high temperature PTFE membrane cartridges are used.

NOTE: Vent filters play a critical role in the design of tank systems. Stainless steel tanks are susceptible to collapse if subjected to vacuum conditions. It is critical to properly size the filter to allow the air flow required at the maximum rate of tank outflow with a pressure drop well below the vacuum rating of the tank. Improper sizing can result in permanent tank damage.

If a large, flexible, collapsible bag is used instead of a tank, the bag will expand and collapse without the need to allow air into the container. Vent filters are not required for flexible bag containers.

Figure 4 – CPF offers a full line of hydrophilic membrane-based cartridge and capsule filters for sterilizing filtration and bioburden reduction.

Figure 5 – The PTM or PTM/HT filters from CPF protect tank contents using hydrophobic PTFE membrane.
References


United States Pharmacopeia 35, United States Pharmacopoeial Convention, Inc. 12601 Twinbrook Parkway, Rockville, MD (2012)


All Critical Process Filtration filter media discussed in this guide are available in cartridge and capsule configurations. Cartridges are available in 5", 10", 20", 30" and 40" nominal lengths. Capsules are available in 2", 5", 10", 20" and 30" nominal lengths. All flat sheet depth media and membranes are available in disc filter format in 13mm, 25mm, 47mm, 90mm and 142mm diameters.

Filter Media Options for Cell Culture Media and Buffer Preparation

<table>
<thead>
<tr>
<th>Process Area</th>
<th>Filter Application</th>
<th>Filter Function</th>
<th>Critical Process Media*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioburden Control and Sterilizing</td>
<td>Bioburden Reduction</td>
<td>Remove most bacteria from the stream to help meet solution quality requirements</td>
<td>BC, CWPS, PVWL, NM, PS</td>
</tr>
<tr>
<td></td>
<td>Sterilizing Filtration</td>
<td>Protect downstream processes from bacteria in solutions</td>
<td>PS, NM</td>
</tr>
<tr>
<td></td>
<td>Tank Vent Filtration</td>
<td>Remove particulate and bacterial contaminants from air entering storage tanks during emptying to help assure solution quality</td>
<td>PVWB, TM</td>
</tr>
</tbody>
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*Media Codes
BC = High Capacity Blended Cellulose Membrane
NM = Nylon 6,6 Membrane
PVWB = High Capacity Hydrophobic PVDF Membrane
CWPS = High Capacity PES Membrane
PS = Polyethersulfone Membrane
TM – PTFE Membrane

Housings
Critical Process Filtration can provide housings for all cartridge filters. Sanitary housings are available for 1, 3, 6, 8, 12 and 21 cartridges from 10" to 40" long.

Visit our website at www.criticalprocess.com or contact Critical Process Filtration for more information, application assistance and access to datasheets for all of our products.