Care and Use Procedures

Minimate™ TFF Capsule

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**Introduction**

The Minimate™ tangential flow filtration (TFF) capsule is the central part of a system for performing concentration or diafiltration on solutions of biomolecules. It is available with low protein binding Omega™ ultrafiltration membranes in several different molecular weight cutoffs. The membranes are encased in a polypropylene housing with luer connectors on the feed, retentate, and filtrate ports for easy connection to a pump and accessories.

- For Laboratory and process development applications
- Ideal for evaluation, process development, process optimization, and validation studies
- Scaleable to LV Centramate™ and Centramate™ systems
- Easily connected up to Minim™ System or peristaltic pumps
- Integrity tested and testable by user
- All plastic construction

**Applications**

Tangential flow filtration is a rapid and efficient method for the concentration or diafiltration of biomolecules. It can be used in a wide range of applications.

- Concentrate and desalt proteins, peptides, or nucleic acids (DNA, RNA, oligonucleotides)
- Recover antibodies or recombinant proteins from clarified cell culture media
- Process metal sensitive enzymes and molecules
- Separate (fractionate) large from small biomolecules
- Recover or remove viruses from solutions
- Prepare samples prior to column chromatography
- Concentrate samples after gel filtration
- Replace dialysis applications
- Depyrogenate water, buffers, and media solutions

**Product Information**

The Minimate TFF Capsule comes with threaded luer plugs in each of the 4 ports. A fitting package with the following components is included.

**Fittings Package**

Part No. 88216 includes:

- A tubing, 3.2 mm (1/8") i.d., 120 cm (4 ft)
- B Male luer-to-hose-barb fittings, 3.2 mm (1/8"), 2
- C Female luer-to-hose-barb fittings, 3.2 mm (1/8"), 2
- D Tubing clamps, 4
- E Retentate screw clamp, 1
- F Adhesive Strip (hook and loop), 1

**Instructions For Use**

NOTE: Read about the capsules operating limits in the specification section of Appendix B of this manual.

**Required Equipment**

- **Pump**
  
  A peristaltic pump or equivalent with variable speed control capable of delivering a constant flow rate from 10 to 100 mL/min/capsule at pressures up to at least 2 bar (30 psi). The peristaltic pump head should be sized to accept 1.6 mm (1/16 in.) or 3.2 mm (1/8 in.) i.d. tubing. The Minimate fitting kit includes 3.2 mm (1/8 in.) i.d. tubing.

- **Feed reservoir**
  
  Appropriately sized for the volume of sample or flushing solutions, e.g. 100 mL or 500 mL. Using a reservoir where liquid is drawn from the bottom allows the sample to be concentrated to a smaller volume due to the reduced hold-up volume

**Recommended Equipment**

- **Pressure measuring device**
  
  Pressure gauge or transducer and display. At least one is recommended on the feed port. Additionally, one on both the retentate and filtrate ports may be added.

- **Magnetic stir plate and stir bar**
  
  Provide adequate mixing in the reservoir
• Stop watch
  Measure elapsed time when determining flow rates (crossflow and filtrate flux rate)
• Pipettes and sample tubes
  Collect samples for analysis
• Beakers / reservoirs
  To hold / collect sample, waste, etc.
• Graduated cylinders
  For accurately determining collected volumes.

Installation
1. Remove the caps from the feed and retentate ports of the Minimate TFF capsule.
   **Note:** Do not discard caps. They are required for storage.
2. Screw a male luer-to-hose-barb connector (included) into each of the feed / retentate ports.
3. Cut a piece of tubing 3.2mm (1/8") i.d., long enough to reach from the feed reservoir, through the pump head to the capsule.
   **Note:** Keep tubing lengths as short as possible to reduce system hold-up volume.
4. Connect the tubing to the hose-barb on one of the feed ports. Install the tubing in the pump head. Put the other end of the tubing into the reservoir.
   **Note:** If a pressure gauge or transducer is used, connect the tubing to the pressure device. Then connect the pressure device as close as possible to the feed port using suitable connectors.
   **Note:** Feed and retentate ports are interchangeable. Depending on the orientation of the capsule, choose the port that is at the lowest elevation as the feed port. This allows for air to be easily expelled when liquid is pumped through the capsule.

The recommended crossflow for the Minimate TFF capsule is 30-40 mL/min.
5. Cut another piece of tubing, long enough to return from the retentate port to the sample reservoir.
6. Attach the tubing to the retentate hose-barb and put the other end in the reservoir. (Again, if a pressure gauge or transducer is used, the tubing connects to the pressure device, which must then be connected to the retentate port.)
7. Place the retentate screw clamp on the retentate tubing close to the retentate port (after the pressure gauge if installed). Secure in place but do not tighten to restrict the tubing.
8. Remove one of the filtrate caps.
9. Attach a female luer-to-hose-barb fitting to one of the filtrate/vent ports.

**Note:** Depending on the orientation of the capsule, choose the filtrate port that is at the highest elevation. This allows air to be completely expelled from the filtrate side of the membrane. The filtrate channel can be drained easily by opening the other filtrate port as a vent.

10. Attach a piece of tubing to the filtrate hose barb.
11. Install a tubing clamp over each piece of tubing where it connects to the hose barb. Pinch the clamp to tighten. Make sure the tubing is secure and does not easily pull off the hose barb.

**Figure 1**
Typical Minimate™ TFF Capsule set-up

The pump is connected with tubing between the process sample reservoir and the feed port on the Minimate TFF capsule. Tubing connected to the retentate port is returned to the reservoir. This allows flow of product across (tangential to) the membrane surface to create a sweeping action that helps prevent membrane fouling by reducing buildup of particles and product on the surface. Pressure in this flow path, generated by the flow stream through the channel and by restricting the tubing connected to the retentate port with an adjustable clamp, is the driving force that creates liquid flow through the membrane (filtrate flow rate). The average pressure applied to the membrane is referred to as the transmembrane pressure (TMP). Increasing the TMP increases the filtrate flow rate, up to a point. Applying too much pressure will foul the membrane and reduce filtrate flow rate. Controlling the TMP helps regulate the filtrate flow rate.

Tubing connected to one of the filtrate ports serves to carry the filtrate (permeate) away from the device. The other filtrate port can be used as a vent for draining all liquid in the tubing after the process is finished.
Calibrate Feed Pump

Note: If the pump does not have a digital read out of flow rate, it may be useful to calibrate the pump. This makes it easier to set flow rates.

Connect tubing to the pump and put both ends in a reservoir with water. Prepare a pump curve by measuring flow rate at different settings. Take measurements at 5 or more settings. Record setting and flow rate. This will allow you to easily adjust flow rates for the following procedures.

Table 1
Pump Calibration

<table>
<thead>
<tr>
<th>Pump Setting</th>
<th>Flow Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Warnings are given in the following procedures not to exceed a feed pressure of 2 bar (30 psi). This warning is related to the pressure rating of the tubing. If it is ascertained that the tubing being used can withstand higher pressures and the tubing clamps have been properly installed, higher pressures can be used. It is strongly recommended that the system be tested at the higher pressures with water before using any potentially dangerous solutions, i.e. caustic sanitizing agents. Do not exceed the pressure rating of any component in the system.

Flushing

The Minimate TFF Capsule contains traces of glycerin (humectant) and preservative (biocide). The humectant insures that the membrane will easily accept water and allow high flux, and the preservation combats microbial growth during storage. These compounds should be flushed out before use. More rigorous flushing and sanitization may be required for more critical uses, while less flushing may be acceptable if the intended use is less critical. If the Minimate TFF Capsule has previously been used, cleaned and equilibrated in a storage solution, the same preconditioning procedure may be used. Follow these steps to flush out the storage solution.

STEP 1

Place the tubing from the suction side of your pump (feed) into about 1 liter of 0.2 um-filtered deionized water or water for injection (WFI). Place the tubing from the retentate and filtrate ports into a drain or waste container.

STEP 2

Adjust pump to deliver a flow rate of about 40 mL/min. Slowly tighten retentate screw clamp to increase backpressure. This will increase flow through the filtrate lines. Tighten clamp until filtrate flow rate is approximately equal to the retentate flow rate or until feed pressure reaches 2 bar (30 psi). Do not exceed a feed pressure of 2 bar (30 psi). Pump at least 250 mL of the water through the retentate tubing and 250 mL through the filtrate tubing to the drain (>500 mL total).

If the Minimate TFF capsule will not be sanitized prior to use, it is recommended that this step is repeated to further remove the glycerin and preservative.

STEP 3

When flushing is almost complete, open the vent port and collect at least 10 mL of filtrate. A small beaker or test tube can be used to collect the liquid. Replace the vent cap when finished.

A three-port valve with luer connectors may be attached to the drain port. A short piece of tubing attached to a luer-to-hose-barb fitting can be connected to one of the valve port. The valve can then be turned to drain the liquid into the beaker or close off the port.

Sanitization (Recommended for critical applications)

If required, the Minimate TFF Capsule may be sanitized prior to use. Sanitization is recommended whenever full compliance with USP Biological Reactivity Test In Vitro <80> is required. Refer to the MSDS for proper handling and safety precautions when working with caustic solutions.

The following solutions can be used to sanitize and depyrogenate the capsule.

1. 0.1 – 0.5N NaOH @ 35 – 45 ºC
2. 0.1 – 0.5N NaOH + 200 ppm NaOCl @ 35 – 45 ºC
3. 200 – 400 ppm NaOCl (pH 6 – 8) @ 25 – 45 ºC
   (Should not be used if any metal parts come in contact with the solution.)

Sanitization is particularly important if the capsule has been previously used, cleaned and stored.

STEP 1

Add about 200 mL of sanitizing solution into a reservoir. Place feed tubing into reservoir. Place the tubing from the retentate and filtrate ports into the reservoir.

STEP 2

Adjust pump to deliver about 50 mL/min. Slowly tighten clamp on retentate tubing until filtrate flow rate is approximately 25% of the retentate flow rate. Do not exceed a feed pressure of 2 bar (30 psi). If filtrate flow rate is already >25% of retentate flow rate, proceed to next step.
STEP 3
Circulate the sanitizing solution for about 45 minutes to 1 hour.

STEP 4
Flush out the sanitizing solution with water. Follow the flushing procedure given above.

Normalized Water Permeability (NWP) (Optional)
This procedure is strongly recommended if the Minimate TFF Capsule will be reused. The water permeability (filtrate flow rate per unit of applied pressure) is a measure of performance of the “original” clean membrane. The effectiveness of the cleaning protocol, i.e. membrane recovery, can be evaluated by comparing the NWP of the membrane after cleaning to the “original” NWP.

\[
\text{Membrane recovery} = \frac{\text{NWP (after Cleaning)}}{\text{NWP (Original)}} \times 100\%
\]

The procedures for determining NWP is given in Appendix A

System Hold-up Volume (Optional)
The System Hold-Up Volume is the total volume contained within the feed/retentate flow path of the TFF system. Most of this volume is recoverable. The Minimum Working Volume is the system hold-up volume plus a minimum volume of liquid that must remain in the bottom of the feed reservoir at the operating flow rate in order to prevent air from being drawn into the cassette system. Increasing the cross flow rate, increases the volume of product required in the bottom of the feed reservoir, in order to prevent air from getting drawn into the pump. The minimum working volume limits the maximum concentration factor achievable. Knowing the minimum working volume, you can calculate the minimum starting volume required to achieve a desired concentration factor.

\[
\text{Minimum Starting Volume} = \text{Minimum Working Volume} \times \text{Factor}
\]

If the actual starting volume is less than this value it will not be possible to reach the concentration factor.

Reservoir design significantly affects the minimum volume required to prevent air from getting into the system. The Minim System 500mL reservoir was especially designed to minimize the working volume in the system.

See Appendix A for a procedure to determine the minimum working volume.

Integrity Test (Optional)
An integrity test is performed to identify any gross defects in the device membrane or seals that could cause product loss prior to use. Capsules are 100% integrity tested after manufacturing. Therefore it is not necessary to perform an integrity test on a new Minimate TFF Capsule. However, for critical applications or if the capsule will be reused, it is recommended that an integrity test be performed to confirm integrity both before and after use.

Several procedures for performing an integrity test (qualitative and quantitative) are given in Appendix A.

Buffer-Conditioning (Optional)
The buffer-conditioning step is used to precondition the Minimate™ TFF Capsule into the sample buffer (equilibrating solution) before the sample is added. This adjusts the pH and prevents the sample from being diluted with water, which may lower the ionic strength and possibly cause precipitation of product or other sample components. It is also used to remove air bubbles from the system and equilibrate the system components and fluid to operating temperature.

STEP 1
Add about 100mL of buffer solution into the reservoir. Place feed tubing into reservoir. Place the tubing from the retentate and filtrate port(s) into the reservoir.

STEP 2
Adjust pump to deliver about 50mL/min. Slowly tighten clamp on retentate tubing until filtrate flow rate is approximately 25% of the retentate flow rate. If filtrate flow rate is already >25% of retentate flow rate, proceed to next step. Do not exceed a feed pressure of 2 bar (30psi).

STEP 3
Circulate the buffer solution for 5-10 minutes.

STEP 4
Place the tubing from the retentate into a drain. Slowly start the pump and run until the liquid in the reservoir just reaches the bottom. DO NOT ALLOW AIR TO BE DRAWN INTO THE TUBING.

Concentration/Diafiltration
Refer to the following application notes:

- Introduction to Tangential Flow Filtration for Laboratory and Process Development Applications, PN33213
- Diafiltration: A Fast, Efficient Method for Desalting or Buffer Exchange of Biological Samples, SD1599

A TFF Process Data Sheet is included at the end of the Appendix B for use in recording experimental conditions.
**Product Recovery**

Following the concentration/diafiltration process, the product must be recovered from the system.

**Product in Concentrate**

After processing, a significant portion of the product is on the membrane in the form of a “gel layer” and needs to be recovered back into the solution before the system is drained. Recirculating fresh buffer can recover most of this gel layer, but may significantly dilute the product that you just concentrated. The following procedure can improve recovery without significant dilution. The actual procedure may have to be varied depending on TFF system configuration.

**STEP 1**

Following concentration/diafiltration, open the retentate valve and close off the filtrate line with a valve or screw clamp. Adjust the pump to give a retentate flow rate of 40-50 mL/min. Circulate the product for 5-10 minutes.

**STEP 2**

Stop the pump. Put the retentate tubing into a collection vessel. Start the pump and slowly pump out the product into the collection vessel. Stop the pump just before the volume in the reservoir reaches the bottom. Add to the reservoir a volume of buffer equal to the system hold-up volume. Pump out the product into the collection vessel stopping just as the liquid level reaches the bottom of reservoir. (This method displaces most remaining product left in the cassettes and hardware.) Record the volume collected.

**STEP 3**

Add just enough volume of buffer to the feed reservoir to allow circulation without pulling in air. Circulate for 10 minutes to try and recover additional product. The remaining liquid in the system can be pumped out into a separate container by allowing air to be pumped through capsule to displace it. A decision can then be made whether to combine this volume with the main product.

**Product in Filtrate**

If the product is in the filtrate, raise the vent port so it is higher than the collection vessel. Make sure the end of the filtrate tubing is placed in the vessel. Open the vent port and allow any remaining filtrate in the capsule and tubing to be drained.

**Cleaning**

If the Minimate™ TFF Capsule is to be reused, it must be cleaned after use and properly stored. Recommended cleaning solutions and protocols are given in Appendix A.

**Storage of Minimate TFF Capsules**

The objective for proper storage is to ensure the membranes remain wet and to prevent microbial growth during the time the membrane cassettes are not being used.

Recommended storage agents for Minimate TFF Capsules.

**Storage Agents:**

<table>
<thead>
<tr>
<th>PERIOD</th>
<th>SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3 days</td>
<td>Sterile water or saline solution</td>
</tr>
<tr>
<td>&lt;6 months</td>
<td>0.05N - 0.1N NaOH</td>
</tr>
<tr>
<td>&gt;6 months</td>
<td>15% glycerin + 0.05% sodium azide</td>
</tr>
</tbody>
</table>

Recommended storage temperatures: 4°C - 15°C (optimal), 25°C (maximum). Do not freeze

**Disposal of Minimate TFF Capsule**

It is recommended that after use, and prior to disposal, the Minimate TFF Capsule be flushed with clean water, sealed into an autoclave bag, and autoclaved at either 121°C for 30 minutes, or at 135°C for 15 minutes to sterilize. Dispose of the Minimate™ TFF Capsule in the bag, and do not open after autoclaving.

**DO NOT** seal the inlet and outlet connectors of the Minimate TFF Capsule prior to autoclaving as this will cause a build up of pressure, which may rupture the device.
Appendix A

Normalized Water Permeability

Determining the Normalized Water Permeability (NWP) for the Minimate TFF Capsule

Water permeability is a function of the hydraulic resistance of the membrane at a specified transmembrane pressure (TMP). It is related to the pore size, pore depth, and number of pores per unit area. It is significantly affected by temperature. Water permeability can be used as a standard upon which to measure the effectiveness of a cleaning regime after sample processing.

The original NWP of the Minimate TFF capsule is essential to calculate as it is used as the basis to determine membrane recovery, i.e., how effectively the membrane was cleaned.

This procedure should be performed with all new Minimate TFF capsules after the flushing and sanitization steps have been performed. The original NWP is determined by plotting the water filtrate flux rates at several transmembrane pressures, typically 0.3 - 1 bar (5 - 15 psig) for UF membranes. From the graph, the original NWP is calculated at 0.7 bar (10 psi). This value is chosen for ease of calculation.

Water quality should be “Water for Injection” (WFI) or at minimum 0.2 µm filtered DI water. Calculated water permeability rates are “normalized” to a temperature of 20 °C by using a temperature correction factor (TCF 20 °C) given in Table 3, page 8.

For Minimate TFF capsules that are reused from this point on, the NWP needs only be measured and determined at a TMP of 0.7 bar (10 psi).

The NWP should be measured before processing and once again after cleaning. An example for determining NWP is shown below.

To determine the NWP, a pressure gauge is required on the feed and retentate ports. Filtrate pressure should be zero (0) provided there are no restrictions on the filtrate line and that the filtrate line is open to atmosphere.

Recirculate the filtrate and retentate streams back to the feed reservoir to minimize the volume of water needed.

Removing air from the retentate

All air must be removed from the retentate channel before determining the NWP, as air bubbles will reduce the effective filtration area, resulting in low NWP values.

Turn on and increase the pump speed to generate a retentate flow rate of 80-100 mL/min. Stop and restart the pump several times. Observe whether any air is expelled from the retentate line when the pump is restarted. If no air is observed, turn off the pump and proceed to the next step.

Determining the “original” normalized water permeability.

1. Tighten the retentate clamp to completely restrict retentate flow. The filtrate line should be open.
2. Adjust the feed flow rate to generate a TMP of approximately 0.33 bar (5 psi).
3. Measure the filtrate flow rate and calculate the flux rate in LMH (liters/m²/h).
4. Adjust the feed flow rate to give a TMP of approximately 0.67 bar (10 psi).
5. Measure the filtrate flow rate and calculate the flux rate in LMH (liters/m²/h).
6. Adjust the feed flow rate to give a TMP of approximately 1.0 bar (15 psi).
7. Measure the filtrate flow rate and calculate the flux rate in LMH (liters/m²/h).
8. Plot Filtrate Flux Rate vs. TMP. Draw a straight line from zero that best fits the data between 0.3 - 1 bar (5 to 15 psig). From the curve determine the water flux rate at 0.7 bar (10 psi).

The following table may be used as an example for recording data and calculations.
Table 2.
Table for recording data and calculating NWP

Measurement Temperature: \________°C, TCF \________

<table>
<thead>
<tr>
<th>Feed Pressure psi/bar</th>
<th>Retentate Pressure psi/bar</th>
<th>Filtrate Pressure psi/bar</th>
<th>TMP psi/bar</th>
<th>Filtrate Flow Rate mL/min</th>
<th>Filtrate Flux Rate mL/min/cm²</th>
<th>Filtrate Flux Rate LMH</th>
<th>Water Permeability LMH/TMP</th>
<th>NWP LMH/TMP @ 20°C</th>
</tr>
</thead>
</table>

\[ \text{TMP} = \left( \frac{P_{\text{feed}} + P_{\text{retentate}}}{2} - P_{\text{filtrate}} \right) \] (Assume \( P_{\text{filtrate}} \) is “0” if no filtrate pressure gauge)

\[ \text{Filtrate Flux Rate (mL/min/cm}^2\text{)} = \frac{\text{Filtrate Flow Rate (mL/min)}}{\text{membrane area of 50 (cm}^2\text{)}} \]

To convert mL/min/cm² to LMH

\[ \text{LMH} = \frac{\text{mL/min/cm}^2}{1000 \times 60 \times 10,000} = \text{mL/min/cm}^2 \times 600 \]

\[ \text{Filtrate Flux Rate (LMH)} = \frac{\text{Filtrate Flux Rate (mL/min/cm}^2\text{)}}{600} \]

\[ \text{Water Permeability (LMH/TMP)} = \frac{\text{Filtrate Flux Rate (LMH)}}{\text{TMP}} \]

\[ \text{NWP (LMH/TMP @ 20 °C)} = \text{Filtrate Flux Rate (LMH)} \times \text{Temperature Correction Factor (TCF)} \text{ at operating temperature} \]

Table 3
Temperature Correction Factors (TCF 20°C) for determining Normalizing Water Permeability

<table>
<thead>
<tr>
<th>T °C</th>
<th>TCF 20 °C</th>
<th>T °C</th>
<th>TCF 20 °C</th>
<th>T °C</th>
<th>TCF 20 °C</th>
<th>T °C</th>
<th>TCF 20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>1.271</td>
<td>21</td>
<td>0.978</td>
<td>31</td>
<td>0.781</td>
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<td>0.641</td>
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<td>12</td>
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<td>0.955</td>
<td>32</td>
<td>0.765</td>
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<td>0.629</td>
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<tr>
<td>13</td>
<td>1.202</td>
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<td>33</td>
<td>0.749</td>
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<tr>
<td>14</td>
<td>1.169</td>
<td>24</td>
<td>0.911</td>
<td>34</td>
<td>0.734</td>
<td>44</td>
<td>0.607</td>
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<tr>
<td>15</td>
<td>1.139</td>
<td>25</td>
<td>0.890</td>
<td>35</td>
<td>0.719</td>
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<td>0.596</td>
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<td>16</td>
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<td>0.871</td>
<td>36</td>
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<td>46</td>
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<tr>
<td>17</td>
<td>1.081</td>
<td>27</td>
<td>0.851</td>
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<td>0.692</td>
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<tr>
<td>18</td>
<td>1.053</td>
<td>28</td>
<td>0.833</td>
<td>38</td>
<td>0.678</td>
<td>48</td>
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</tr>
<tr>
<td>19</td>
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<td>29</td>
<td>0.815</td>
<td>39</td>
<td>0.665</td>
<td>49</td>
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</tr>
<tr>
<td>20</td>
<td>1.000</td>
<td>30</td>
<td>0.798</td>
<td>40</td>
<td>0.653</td>
<td>50</td>
<td>0.547</td>
</tr>
</tbody>
</table>
EXAMPLE

Determination of NWP on a Minimate TFF Capsule

Water filtrate flux rates were measured for a Minimate TFF Capsule at TMP's of 5, 10, and 15 psig. The temperature of the water was 16°C. Determine the original normalized water permeability (NWP).

Table 4
Data and Calculations from Example

<table>
<thead>
<tr>
<th>Feed Pressure psi/bar</th>
<th>Retentate Pressure psi/bar</th>
<th>Filtrate Pressure psi/bar</th>
<th>TMP psi/bar</th>
<th>Filtrate Flow Rate mL/min</th>
<th>Filtrate Flux Rate mL/min/cm²</th>
<th>Filtrate Flux Rate LMH</th>
<th>Water Permeability LMH/TMP</th>
<th>NWP (LMH/TMP @ 20°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>4.6</td>
<td>0.092</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>9.2</td>
<td>0.184</td>
<td>110</td>
<td>11</td>
<td>12.2</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>13.8</td>
<td>0.276</td>
<td>165</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Water Permeability** = 110 LMH @ 10 psig = 11.0 LMH/psig

**Normalized Water Permeability** = 11.0 LMH/psig x TCF 20 °C where TCF 20 °C = 1.109

(Correcting water permeability for temperature; from 16 °C to 20 °C)

**Temperature correction factor from Table 3 for 16 °C** = 1.109

**Normalized Water Permeability** = 11.0 x 1.109 = 12.2 LMH/psig
**System Hold-up Volume**

The **system hold-up volume** is the total volume contained within the feed/retentate flow path of the TFF system. Most of this volume is recoverable. The **Minimum Working Volume** is the system hold-up volume plus a minimum volume of liquid that must remain in the bottom of the feed reservoir at the operating flow rate in order to prevent air from being drawn into the cassette system. At a higher cross flow rate, a greater volume of product is required in the bottom of the feed reservoir, to prevent air from getting drawn into the pump. The minimum working volume limits the maximum concentration factor achievable. Knowing the minimum working volume, you can calculate the minimum starting volume required to achieve a desired concentration factor.

\[
\text{Minimum Starting} = \frac{\text{Minimum Concentration Volume}}{\text{Working Volume}} \times \text{Factor}
\]

If the actual starting volume is less than this value it will not be possible to reach the concentration factor. Reservoir design significantly affects the minimum volume required to prevent air from getting into the system. The Minim System 500mL reservoir was especially designed to minimize the working volume in the system.

**Procedure to determine System Hold-up Volume**

It is convenient to perform this procedure following the determination of water permeability and before performing air integrity measurements. The Minimate TFF capsule and lines should already be filled with water and you will need to drain the feed/retentate flow path for the air integrity test anyway.

1. Clamp off the filtrate line.
2. Open the retentate screw clamp.
3. Pump down water in reservoir until volume is just above bottom of reservoir; then stop pump. (Do not allow air to be drawn into the tubing.)
4. Carefully transfer the retentate line into a 25mL graduated cylinder. (On Minim System, turn retentate bypass valve to drain and direct tubing into graduated cylinder.
5. Turn on pump and allow remaining liquid to be pumped out.
6. Record the volume. This volume is the “system hold-up volume”.

**Procedure to determine Minimum Working Volume**

1. Add water to the reservoir.
2. Direct retentate and filtrate lines into feed reservoir. Open the retentate clamp and close the filtrate clamp.
3. Adjust pump to deliver the operating retentate flow rate that will be used for the process.
4. Direct the retentate line to drain. Watch the water in the reservoir. When the water reaches a level where it appears that air is just about to get pulled up into the feed tubing, immediately return the retentate tubing back into the feed reservoir. Continue to circulate the water for a minute to confirm that no air is drawn into the tubing. If any air is drawn in, add water back into the reservoir until no more air is drawn into the tubing.
5. Direct the retentate tubing into a graduated cylinder and allow all the liquid to be pumped out into the cylinder.
6. Stop the pump. If any liquid remains in the reservoir, add it to the cylinder.
7. Record the volume in the cylinder. This is the minimum working volume for the system.

**Minimate TFF Capsule Integrity Test**

Forward Flow Measurement is a quantitative test that measures the rate of air diffusing through the wetted membrane or passing through seal defects at a given pressure. Air diffusion rates can be performed on cassettes wetted with water or buffer solution.

Capsules are 100% integrity tested after manufacturing. Therefore it is not necessary to perform an integrity test on a new Minimate TFF Capsule. However, for critical applications or if the capsule will be reused, it is recommended that a forward flow air integrity test be performed to confirm integrity before and after use.

The **forward flow integrity test** detects system or membrane leaks. It does not provide a means to determine retention characteristics of the membrane.

A simple, quick and inexpensive test protocol is described below which can be performed without expensive measuring equipment. The protocol is performed after the capsule has been preconditioned and flushed with water in order to completely wet-out the membrane. A pressure gauge at the feed port is required. The pump must be self-priming, able to pump air at pressures to 30psi. The Minim System and most peristaltic pumps are suited for this procedure.
WATER DISPLACEMENT METHOD (QUANTITATIVE)

The first step is to thoroughly wet the membrane with water assuring that each pore is filled with liquid. Once the membrane is thoroughly wetted, the feed/retentate flow path is drained. A graduated cylinder is filled with water and carefully turned upside down in a beaker full of water. The end of the filtrate tube is placed inside the graduated cylinder. The retentate port is then closed off. The system is pressurized to about 10 psig (0.7 bar) through the device feed port. Air will diffuse through the membrane, exiting through the filtrate tubing and into the graduated cylinder. The amount of air displaced over time is measured in mL/min. This is the air diffusion (forward flow) integrity test value.

Initially water in the filtrate line will be displaced. Wait until air starts to flow before taking any measurement, as any water left in the feed channel will first pass across the membrane. The membrane creates a barrier to air flow, not water flow.

Figure 2
Set-up for Air Integrity Testing of Minimate TFF Capsule

This protocol describes performing the procedure using a peristaltic pump to generate airflow. For more accurate testing, a regulated, compressed air or nitrogen supply should be used.

Forward Flow Air Integrity Test Procedure:

1. Empty the feed reservoir.
2. Open retentate and filtrate screw clamps and pump out liquid from feed and retentate tubing.
3. Fill a 400 mL or larger beaker and a 25 mL graduated cylinder with water.
4. Invert the full graduated cylinder into the large beaker. (Place a piece of paper or Parafilm over the mouth of the cylinder. Turn the cylinder over and remove the piece of paper after the mouth of the cylinder is below the water level in the beaker.) The cylinder should remain filled with water.
5. Place the end of the filtrate tubing into the beaker. The filtrate tube should be open.
6. Slowly turn up the pump speed to give a retentate flow rate of 40 mL/min. Tighten the retentate screw clamp to apply a feed pressure of 10 psi.
7. Watch for bubbles emerging at the end of the filtrate tube. When the flow seems to be consistent, place the end of the filtrate tubing into the inverted cylinder. The cylinder can now rest on the bottom of the beaker. Make sure that the filtrate tubing is not crimped under the cylinder mouth.
8. Measure the amount of air entering the graduated cylinder for sixty seconds (mL/min). Compare to integrity specifications for the Minimate TFF capsule.
9. If no air bubble reaches the cylinder within 3-5 minute, the device is most likely within specifications. (Calculate the volume in the filtrate tubing and compare to the allowable airflow rate to see if sufficient waiting time has been allowed.)
PRESSURE-HOLD TEST METHOD (QUALITATIVE)

This procedure is a simple, quick method to test the integrity of a capsule and system. It involves pressurizing the capsule and then watching for a pressure decline. A drop in pressure indicates air leakage. The method can be used to check there are no leaks in connections and to confirm there are no gross defects in the capsule membrane.

Forward Flow Air Integrity Test Procedure:
1. Empty feed reservoir.
2. Open retentate screw clamp and pump out liquid from feed reservoir.
3. Clamp both the retentate and filtrate lines.
4. Slowly increase the pump speed to build up the pressure to 1.6 bar (25 psi).
5. Stop the pump.
6. Clamp the feed tubing so air cannot flow backwards through the pump head.
7. Slowly open the filtrate screw clamp to relieve the pressure.
8. Observe the pressure on the feed pressure gauge. After an initial drop when the filtrate clamp is opened, the pressure should hold steady. (If pressure drops below 0.7 bar [10 psi] when the filtrate valve is opened, remove the pump clamp and start the pump to bring the feed pressure to about 0.7 bar [10 psi]. Stop pump and reapply clamp. If the pressure holds for 2 minutes, the cassette integrity should be good. On a defective cassette, the pressure would drop the 10 psi in a few minutes.
9. Observe the filtrate line. When the filtrate clamp is first opened, some liquid will be displaced. This is liquid which remained on the feed side and which is forced through the membrane. (Liquid will pass freely through the membrane, but not the air.) Liquid on the filtrate side will continue to be displaced if there is airflow through the membrane or seals until all the liquid in the tubing has been displaced. If you see a lot of air bubbling through the filtrate line, there could be an integrity failure. In this case a quantitative integrity test should be performed.

Flushing and Cleaning After Use

Recommended cleaning agents for Omega membranes in the Minimate™ TFF capsule.

Cleaning Solutions:
• 0.5N NaOH at 35-45 °C
• 0.1-0.5N NaOH + 200-400 ppm NaOCl at 35-45 °C
• 0.1N nitric, acetic or phosphoric acid

Post Treatment of Minimate TFF Capsule

Before introducing the cleaning solution into the membrane system, remaining product and contaminants should first be flushed free using either buffer or spent filtrate. The use of a buffered flush will eliminate possible solubility changes. Spent filtrate, assuming the product is in concentrate, eliminates the need for fresh buffer or water to be used to flush the system prior to introducing the cleaning agent, eliminating additional fouling concerns.

To flush the system, the retentate is directed to drain. For cleaning, the retentate and filtrate lines are returned back to the feed reservoir.

To assure proper flushing, it is essential to obtain high fluid velocities through the system to create sufficient turbulence at all wetted surfaces (membrane, tubing, components, etc.). It is also important to develop a positive pressure profile along the entire membrane path length (feed to retentate). The procedure below will accomplish both requirements.

Note: This procedure requires a screw clamp on both the retentate and filtrate lines.

Flushing the retentate stream of excess matter
1. Direct the filtrate and retentate streams to waste.
2. Add buffer or spent filtrate to feed reservoir.
3. Open the retentate screw clamp and close the filtrate screw clamp.
4. Start the pump and increase speed until retentate flow rate is 50-80 mL./min.
5. Flush about 200mL to drain to assure that a thorough flushing is accomplished.

Flushing the system with water
1. Drain the system
2. Add at least 500 mL of 0.2 um-filtered deionized water or WFI. Place the tubing from the retentate and filtrate port into a drain or waste container.
3. Adjust pump to deliver a flow rate of about 40mL/min. Slowly tighten the retentate screw clamp until the filtrate flow rate is approximately equal to the retentate flow rate. Do not exceed a feed pressure of 2 bar (30 psi).
4. Pump at least 250 mL of the water through the retentate tubing and 50 mL through the filtrate tubing to the drain (>500mL total).

Adding and circulating cleaning agent.
1. Drain system. Add the cleaning solution to the feed reservoir.

Note: Cleaning is more effective if solution is warmed to 35-45°C before adding to reservoir.
1. Return the retentate and filtrate line back to the feed reservoir.
2. Open the retentate and filtrate screw clamps.
3. Increase the pump speed to deliver 50-80 mL/min through retentate.
4. Adjust retentate clamp to generate a retentate pressure of about 2 bar (30 psi).
5. Run for about 2-3 minutes to flood the membrane with cleaning solution.
6. Open the retentate clamp and close the filtrate clamp.
7. Adjust pump speed to give a retentate flow rate of 50-80 mL/min.
8. Run for 45 - 60 minutes for cleaning.

**Note:** If the cleaning solution gets “dirty” after only a few minutes, flush it out from the system using the following procedure and then immediately add fresh cleaning solution and repeat the cleaning cycle. Do not continue to circulate dirty cleaning solution as it will reduce the effectiveness of the cleaning process.

### Flushing the Cleaning Agent from capsule

1. Direct retentate and filtrate lines to drain.
2. Open retentate and filtrate clamps.
3. Remove feed tubing from cleaning solution.
4. Start pump and run until all solution has been purged from capsule.
5. Drain reservoir and add water for flushing, 0.2 µm filtered deionized water or WFI.
6. Close the filtrate clamp.
7. Turn on the pump and adjust to 50-80 mL/min. Run at least 250mL through retentate to drain.
8. Stop the pump. Open filtrate clamp and then close retentate clamp. Refill reservoir with water if necessary.
9. Start pump and increase flow until the feed pressure is about 2 bar (30 psi). Run until a minimum of 250 mL is flushed through the filtrate to drain.

### Determining the Membrane Recovery for the cleaned Minimate™ TFF Capsule

Membrane recovery is a calculation that defines the efficiency of the cleaning process performed on the membrane capsule. It compares the normalized water permeability (NWP) after cleaning to the "original" NWP. The original NWP was measured when the membrane was first installed and preconditioned (described on page 5).

Water should be waterWFI or at a minimum, 0.2 µm filtered DI water.

All water permeability rates are normalized to a temperature of 20 °C by using a temperature correction factor (TCF 20 °C) in Table 3.

#### Determining the water permeability after cleaning

1. Add water to the reservoir.
2. Return the retentate and filtrate line back to the feed reservoir.
3. Open the retentate and filtrate screw clamps.
4. Increase the pump speed to deliver 80 -100 mL/min through retentate. Stop and restart the pump several times. Observe whether any air is expelled from the retentate line when the pump is restarted. If no air is observed, turn off pump and proceed to the next step.
5. Close the retentate clamp. Adjust the pump flow rate to develop an transmembrane pressure of approximately 10 psig (0.7 bar).

\[
\text{TMP} = \left( \frac{P_{\text{feed}} + P_{\text{retentate}}}{2} \right) - P_{\text{filtrate}}
\]

6. Measure the filtrate flow rate.
7. Calculate the filtrate flux rate in mL/min/cm² and LMH.
8. Calculate the water permeability at the recorded transmembrane pressure.

\[
\text{Water Permeability} = \text{Filtrate Flux Rate (LMH)} \times \text{TMP}
\]

9. Calculate the Normalized Water Permeability using the temperature correction factor (TCF 20°C) in Table 3, page 8.

\[
NWP = \text{Water Permeability} \times \text{TCF 20°C}
\]

10. Calculate the membrane recovery.

\[
\text{Membrane Recovery} = \left( \frac{NWP \text{ after cleaning}}{"original" \ NWP} \right) \times 100\%
\]

If the membrane recovery is less than 75 - 80%, perform another cleaning cycle starting from the step, “Adding and circulating cleaning agent”.

If after the second cleaning the NWP has increased but acceptable recovery has not been achieved, perform another cleaning cycle. If after the second cleaning the NWP has not increased, the cleaning agent or parameters (time, temperature, etc.) may have to be altered.

**NOTE:** Between uses, the capsule is stored in a caustic solution (typically 0.05N - 0.1 NaOH).

During this time, remaining foulants on the membrane may be released and removed from the capsule when it is flushed prior to the next use. Therefore, it is not unusual to find membrane recoveries increase after storage versus values obtained immediately after cleaning.
Appendix B  
Specifications

Materials of Construction

Filter media: Omega membrane (m-polyethersulfone)

Housing:
- polypropylene, glass reinforced
- screens, housing, housing sealing ring, fittings: polypropylene
- Membrane plate/filtrate channels: polyethylene
- Internal gasket: ethylene propylene elastomer

Product Dimensions
- 20cm x 3.8cm x 1.8cm (L x W x H, nominal)
- 8in x 1.5 in x 0.7 in

Effective Filtration Area
- 50 cm²

Product Hold Up Volume (feed/retentate)
- ~1.6mL

Forward Flow Air Integrity Value
- ≤ 7 mL/min at 0.75 bar (10 psi)

Operating Temperature Range
- 5 ºC (41 ºF) to 50 ºC (122 ºF)

Maximum Operating Pressure at 20 ºC (68 ºF):
- 4 bar (400 kPa, 60 psi)

Recommended Crossflow
- 30-40 mL/min (0.6-0.8 Liters/min/ft²)

Connections
- Feed: Female Luer with external thread
- Retentate: Female Luer with external thread
- Filtrate: Male luer with threaded lock ring
**Certification**

Pall Corporation certifies that the Minimate™ TFF Capsule is:

- **100% POST PRODUCTION INTEGRITY TESTED**
- Field integrity testable using pressure hold or forward flow tests
- Materials of construction pass United States Pharmacopeia (USP) Biological Reactivity Test, In Vivo <88> after preconditioning
- In compliance with published product specifications
- Manufactured in ISO9000 certified facilities

The certificate of test provided does not apply to products that have been opened or damaged by conditions outside the control of Pall Corporation.

**Where to Find More Information**

This manual, Minimate TFF Capsule Care and Use Procedures (PN88227) is supplied on the Minimate TFF Capsule CD-Rom (PN88201) enclosed with the product. Many additional accessories are available for this product, including pumps, reservoirs and connectors. For further assistance, please consult with your local Pall representative or distributor.

**Support Literature**

- Minimate TFF Capsule Data Sheet, USD 2218
- Minimate TFF Capsule Quick Start Guide, PN 88200
- Minimate TFF Capsule CD-Rom, PN 88201
- Introduction to TFF for Laboratory and Process Development Applications, PN 33213
- Diafiltration: A Fast, Efficient Method for Desalting, or Buffer Exchange of Biological Samples, PN 33289
- Desalting and buffer exchange by Dialysis, Gel Filtration or Diafiltration PN 33290
- Minim™ System Product Data, USD 2069
- LV Centramate™ Data Sheet, USD 2098
- Centramate™ Cassette Data Sheet, USD 2080
# Pall Life Sciences
## Tangential Flow Filtration Process Data Sheet

<table>
<thead>
<tr>
<th>Sample Identification:</th>
<th>TFF System:</th>
<th>Membrane/Cassettes:</th>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application:</td>
<td></td>
<td>Membrane Area:</td>
<td>Operator:</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Process Time</th>
<th>Feed Temp</th>
<th>Pressure Readings</th>
<th>Flow Rate/Flux Rate</th>
<th>Volume</th>
<th>Conc. Factor</th>
<th>Diafiltrate Volumes</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr:min</td>
<td>°C</td>
<td>Feed Retent. Filtrate TMP Delta P Retent. CFF Filtrate Filtrate</td>
<td>Feed Filtrate Factor</td>
<td>mL mL x</td>
<td>DV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>psi/bar psibar psibar psi/bar psi/bar psi/bar psi/bar</td>
<td>L/min L/min/ft2 mL/min LMH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Initial Water Data

- Filtrate Flux Rate
- Delta P

### Process Data

- Optimization: Yes or No

### Final Water Data

- Filtrate Flux Rate
- Delta P
## TFF Process Data Sheet Column Description

<table>
<thead>
<tr>
<th>COLUMN</th>
<th>UNITS</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process Time</td>
<td>hr:min</td>
<td>Elapsed process time from beginning of concentration or diafiltration process. Either record actual clock time or elapsed time.</td>
</tr>
<tr>
<td>Feed Temperature</td>
<td>°C</td>
<td>Measured temperature of process solution</td>
</tr>
</tbody>
</table>

### Pressure Readings

<table>
<thead>
<tr>
<th>Feed</th>
<th>psi/bar</th>
<th>Feed Pressure (PF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retentate</td>
<td>psi/bar</td>
<td>Retentate Pressure (PR)</td>
</tr>
<tr>
<td>Filtrate</td>
<td>psi/bar</td>
<td>Filtrate Pressure (PP)</td>
</tr>
<tr>
<td>Transmembrane Pressure (TMP)</td>
<td>psi/bar</td>
<td>([PF + PR] ÷ 2) - PP</td>
</tr>
<tr>
<td>Delta P</td>
<td>psi/bar</td>
<td>Pressure differential (PF – PR)</td>
</tr>
</tbody>
</table>

### Flow Rate / Flux Rate

<table>
<thead>
<tr>
<th>Retentate Flow Rate</th>
<th>L/min</th>
<th>Measured retentate flow rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross Flow Flux (CFF)</td>
<td>L/min/ft²</td>
<td>Retentate flow Rate ÷ membrane area (Liters / minute / ft²)</td>
</tr>
<tr>
<td>Filtrate Flow Rate</td>
<td>mL/min</td>
<td>Measured filtrate flow rate</td>
</tr>
<tr>
<td>Filtrate Flux Rate</td>
<td>LMH</td>
<td>Filtrate flow rate ÷ membrane area (Liters / hour / meter²)</td>
</tr>
</tbody>
</table>

### Volume

<table>
<thead>
<tr>
<th>Feed</th>
<th>mL</th>
<th>Remaining Feed Volume = (Starting Volume) - (Total collected filtrate volume) (Starting Volume equals process volume added to reservoir plus hold-up volume if system was filled with liquid.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtrate</td>
<td>mL</td>
<td>Total collected filtrate volume</td>
</tr>
<tr>
<td>Concentration Factor</td>
<td>X</td>
<td>Starting Volume ÷ (Starting Volume - Filtrate Volume)</td>
</tr>
<tr>
<td>Diafiltration Volumes</td>
<td>DV</td>
<td>The number of DV's of diafiltration solution added. 1 DV = Remaining Feed Volume at start of diafiltration.</td>
</tr>
</tbody>
</table>

### Comments

Indicate any observations or information considered important to fully describe process or events.

To convert milliliters /minute to liters/ hour, multiply by 0.06

To convert square feet to square meters, multiply by 0.093
### Ordering Information

**Minimate TFF capsules; Self contained Ultrafiltration Tangential Flow Filtration capsule with Luer Connectors**

<table>
<thead>
<tr>
<th>Product No.</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>OAD65C12</td>
<td>Minimate TFF Capsule w/650 dalton Omega*</td>
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<tr>
<td>OA001C12</td>
<td>Minimate TFF Capsule w/1Kd Omega</td>
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<tr>
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<td>Minimate TFF Capsule w/3Kd Omega</td>
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<tr>
<td>OA070C12</td>
<td>Minimate TFF Capsule w/70Kd Omega</td>
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</tbody>
</table>

<table>
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<th>Product No.</th>
<th>Description</th>
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<tbody>
<tr>
<td>OA100C12</td>
<td>Minimate TFF Capsule w/ 100Kd Omega</td>
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<td>Minimate TFF Capsule w/ 300Kd Omega</td>
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<tr>
<td>OA500C12</td>
<td>Minimate TFF Capsule w/ 500Kd Omega</td>
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<tr>
<td>OA990C12</td>
<td>Minimate TFF Capsule w/ 1000Kd Omega</td>
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<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>88216</td>
<td>Minimate Fittings kit, Contains: Male luer to 3.2 mm (1/8&quot;) hose Barb, Female luer to 3.2 mm(1/8&quot;) hose Barb, 3.2 mm (1/8&quot;) i.d. tubing, Tubing screw clamp, Tubing clamps, Adhesive strips (loop and hook)</td>
</tr>
</tbody>
</table>

### Complementary Products

<table>
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<tr>
<th>Product No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS700X06</td>
<td>3-port valves (6)</td>
</tr>
<tr>
<td>FS700M01</td>
<td>MINIM™ System Pump station with 500mL reservoir, 1.6mm (1/16 in.) i.d. tubing, 3.2 mm (1/8 in.) i.d. tubing, 3 port valves, fittings. Multi-voltage unit. Supplied with detachable power cord.</td>
</tr>
<tr>
<td>FS720M01A</td>
<td>Pressure Display Unit* Display reading in psi,100-120VAC, 60 Hz</td>
</tr>
<tr>
<td>FS720M01B</td>
<td>Pressure Display Unit* Display reading in bar,200-240VAC, 50 Hz</td>
</tr>
</tbody>
</table>

*Three digital displays for Feed, Retentate and Filtrate Pressure (includes 6 pressure transducers).