Affinity Chromatography Media

ProSep®-vA Ultra Media
ProSep-vA High Capacity Media
ProSep-A High Capacity Media
ProSep-rA High Capacity Media

OPERATING INSTRUCTIONS
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**Introduction**

The ProSep-A family of protein A affinity media includes:

<table>
<thead>
<tr>
<th>Media</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ProSep-vA Ultra media</td>
<td>Vegan native protein A immobilized on 700 Å</td>
</tr>
<tr>
<td></td>
<td>Controlled Pore Glass</td>
</tr>
<tr>
<td>ProSep-vA High Capacity</td>
<td>Vegan native protein A immobilized on 1000 Å</td>
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<tr>
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<td>Native protein A immobilized on 1000 Å</td>
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<td>media</td>
<td>Controlled Pore Glass</td>
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<tr>
<td>ProSep-rA High Capacity</td>
<td>Recombinant protein A immobilized on 1000 Å</td>
</tr>
<tr>
<td>media</td>
<td>Controlled Pore Glass</td>
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</table>

ProSep-A affinity adsorbents have been developed to facilitate highly efficient, cost effective purification of both monoclonal and polyclonal antibodies. ProSep-vA Ultra media and ProSep-vA High Capacity media are manufactured using no mammalian products.

ProSep-A media consist of protein A immobilized on porous glass which is permeated by interconnecting pores of uniform and precisely controlled size. ProSep-A media exhibit chemical and mechanical stability over a range of conditions such as low pH, exposure to detergents, buffers of varying ionic strengths, and many organic solvents. ProSep-A media are incompressible, extremely durable, and do not shrink or swell in different solutions. They have a narrow pore size distribution (80% of the pores show a deviation of less than ±10% from the nominal pore diameter) coupled with a large internal surface area.

Proprietary chemistry is used for the immobilization of protein A onto the controlled pore glass and has been developed to satisfy four critical factors:

- orientation of the ligand
- distribution of the ligand
- stability of the immobilized ligand
- minimization of nonspecific surface interactions

The combination of these factors means that ProSep A media are ideally suited for the efficient, cost effective and rapid purification of antibodies directly from clarified bioreactor feedstock, particularly for large scale industrial use.

ProSep-vA Ultra media, the result of an extensive development program, retains the same basic attributes of ProSep-vA High Capacity media but provides even higher binding capacity. It is specifically designed to provide high productivity and low cost of operation for large volume, higher titer feedstocks.
**Intended Use**

The ProSep-A family of media may be used for applications such as:

- purification of monoclonal antibodies (MAbs)
- purification of fusion proteins
- selective purification of antibody fragments
- purification or removal of polyclonal IgG from serum
- separation of IgG subclasses using a stepwise pH gradient

ProSep-A affinity media have been developed specifically for industrial scale purification of monoclonal antibodies where highly efficient purification can be achieved using clarified bioreactor feedstock at physiological pH and salt concentration. No pretreatment such as concentration by ultrafiltration or buffer exchange to increase salt concentration and capacity is required. This reduces time, labor and capital cost and improves retention of biological integrity of the antibody product.

In some cases ProSep A media may also be used to purify antibody fragments (for example, see Kelley et al, 1992).

**ProSep-vA Ultra media or ProSep-vA High Capacity media?**

ProSep-vA Ultra media with its 700 Å pore size and high surface area provides the best combination of capacity and throughput for most antibodies with a molecular weight in the region of 150 kD (IgG’s). For fusion proteins or conjugates where the actual or effective molecular weight is larger than 150 kD, the 1000 Å based ProSep-vA High Capacity media may result in higher dynamic capacity due to the larger pore diameter which provides less hindered mass transfer for the larger molecule.
# Typical Specifications

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Porous glass, Particle size, 75 - 125 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore Size</td>
<td>700 Å, ProSep-vA Ultra media</td>
</tr>
<tr>
<td></td>
<td>1000 Å, ProSep-vA High Capacity media,</td>
</tr>
<tr>
<td></td>
<td>ProSep-A High Capacity media, ProSep-</td>
</tr>
<tr>
<td></td>
<td>rA High Capacity media</td>
</tr>
<tr>
<td>pH range</td>
<td>pH 1 to 9</td>
</tr>
<tr>
<td>Pressure</td>
<td>Incompressible matrix with linear</td>
</tr>
<tr>
<td></td>
<td>pressure/flow rate characteristics.</td>
</tr>
<tr>
<td>Maximum</td>
<td>&lt; 3000 psi (200 bar)</td>
</tr>
<tr>
<td>operating</td>
<td></td>
</tr>
<tr>
<td>Packed Bed</td>
<td>1.3 g/mL</td>
</tr>
<tr>
<td>Density</td>
<td></td>
</tr>
<tr>
<td>Life</td>
<td>ProSep-A media are stable over repeated</td>
</tr>
<tr>
<td></td>
<td>operational cycles when proper cleaning</td>
</tr>
<tr>
<td></td>
<td>protocols are used.</td>
</tr>
<tr>
<td>Protein A</td>
<td>Native protein A from <em>Staphylococcus</em></td>
</tr>
<tr>
<td>Ligand</td>
<td><em>aureus</em>, ProSep-vA Ultra media, ProSep-</td>
</tr>
<tr>
<td></td>
<td>vA High Capacity media, ProSep-A High</td>
</tr>
<tr>
<td></td>
<td>Capacity media</td>
</tr>
<tr>
<td></td>
<td>Recombinant protein A expressed in *E.</td>
</tr>
<tr>
<td></td>
<td>coli*, ProSep-rA High Capacity media</td>
</tr>
<tr>
<td>Binding</td>
<td>ProSep-vA/A/rA High Capacity media</td>
</tr>
<tr>
<td>(Static)</td>
<td>Human IgG ≥ 40mg/mL</td>
</tr>
<tr>
<td>Capacity</td>
<td>Bovine 27 mg/mL</td>
</tr>
<tr>
<td></td>
<td>Goat 17 mg/mL</td>
</tr>
<tr>
<td></td>
<td>Mouse 33 mg/mL</td>
</tr>
<tr>
<td></td>
<td>Porcine 38 mg/mL</td>
</tr>
<tr>
<td></td>
<td>Rabbit 37 mg/mL</td>
</tr>
<tr>
<td></td>
<td>Rat 13 mg/mL</td>
</tr>
<tr>
<td></td>
<td>Sheep 13 mg/mL</td>
</tr>
<tr>
<td>ProSep-vA</td>
<td>Human IgG ≥ 56 mg/mL</td>
</tr>
<tr>
<td>Ultra media</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Typical dynamic capacity for humanized</td>
</tr>
<tr>
<td></td>
<td>monoclonal antibodies ranges from 15-30</td>
</tr>
<tr>
<td></td>
<td>mg/mL for ProSep-vA/A/rA High Capacity</td>
</tr>
<tr>
<td></td>
<td>media and 20-45 mg/mL for ProSep-vA</td>
</tr>
<tr>
<td></td>
<td>Ultra media.</td>
</tr>
</tbody>
</table>
Pressure Drop and Flow Rate

The particle size range and rigid nature of ProSep-A media mean that pressure drop, even at high flow rates is low and that pressure/flow data exhibit a linear relationship, irrespective of column diameter. This ensures that scale-up is straightforward and predictable, even when moving to very large production columns over 1 meter diameter.

The low inherent pressure drop enables high flow rates and/or longer bed lengths to be utilized, increasing flexibility in process design. The predicted pressure drop can be calculated using the following equation.

\[ \Delta P(\text{bar})/L(\text{cm}) = 4 \times 10^{-5} \times V (\text{cm/hr}) \]
Quality Assurance
Each batch of ProSep-A affinity media is tested prior to shipment to determine the binding capacity with standard solutions of human polyclonal IgG. The results of these tests are included in the Certificate of Analysis accompanying each pack.

All ProSep products are manufactured in a facility certified to the internationally recognized standard, BS EN ISO9001:2000.

Precautions and Safety Procedures
1. The ProSep-A family of media is supplied in buffer containing preservative. Do not mouth pipette. The buffer may be harmful if swallowed.

2. Do not expose ProSep-A media to pH outside of the range of 1.0 - 9.0.

First Aid
Eyes  Irrigate thoroughly with water. If discomfort persists obtain medical attention.

Skin  Wash thoroughly with soap and water.

Mouth  Rinse thoroughly with water. In severe cases, obtain medical attention.

Spills  Wear appropriate protective clothing. Carefully mop-up spill and dispose of in accordance with local regulations. Large spills of material should be contained with sand and transferred to salvage containers.

Health and Safety
The Material Safety Datasheet for this product and all reagents used with it should be carefully reviewed prior to beginning work. Work should not commence until the user is thoroughly familiar with the health and safety hazards of all reactants and the hazardous nature of their interactions.
Sample Preparation

Monoclonal Antibodies

Cell culture supernatant, or other bioreactor feedstock, should be clarified to remove cell debris before loading onto the column at pH 7.0 – 8.0 and salt concentration of not less than 0.15M.

Concentration of antibody (e.g. by ultrafiltration) or buffer exchange is not required. Ascites should be diluted 50:50 v/v with 1M glycine/0.3 M NaCl, pH 8.6 buffer and stored at 4 °C for 24 hours prior to removal of any precipitate by centrifugation and/or filtration using a 0.2 µm filter before loading onto the column.

Polyclonal Antibodies

Lipids should be removed from plasma or serum by dialysis at 4 °C against 0.025 M sodium acetate buffer pH 5.2 containing 0.01M NaCl. The centrifuged supernatant should then be dialyzed against or diluted 50:50 v/v with binding buffer (Phosphate Buffered Saline, PBS) and the pH adjusted to pH 7.4. Filter before loading onto the column.

Partial purification of IgG by precipitation using ammonium sulfate or polyethylene glycol is not required.
Column Packing

Due to its low pressure drop and incompressible nature, ProSep-A media can be packed to longer bed lengths than more compressible media types. A well packed column is one where the density of the packed bed is maximized and homogeneous; that is the spaces between the media particles are minimized and uniform. With small diameter laboratory columns, tapping the column during packing facilitates settling. With larger diameter columns, an upward fluidization step prior to flow packing can be used.

ProSep-A media do not exhibit cracking of the column bed, which maybe experienced with soft matrices and are unaffected if accidentally allowed to run dry during operation. ProSep-A media do not expand or contract under differing conditions of ionic strength, or pH.

Note: If the column does dry out, remove trapped air by flowing buffer in an upward direction. Some sintered bed supports may not allow passage of air due to surface tension effects. In such cases, removing and replacing the top flow adapter should solve this.

Packing laboratory and small scale columns
1. Calibrate column to the volume or bed height required. Mark with waterproof marker.
2. Partially fill column with buffer to wet bottom bed support. Close outlet.
3. Remove fines by re-suspending the media then allowing to gravity settle for 15 minutes. Decant any fines. Once “de-fined,” pour the media into the column (typically a 50-60% slurry). Avoid trapping air by pouring down column wall or a glass rod.
4. Open the outlet at the bottom of the column to allow flow and add more ProSep-A media. Continue adding media until the level of ProSep-A media in the column comes up to the desired level.
5. Add further packing buffer. Tap the sides of the column to settle the ProSep-A media. Continue tapping until the bed height remains constant.
6. Add more ProSep-A media and repeat steps 3 – 5 until the media is at the desired level and tapping does not settle the column any longer. Close the outlet of the column.
7. Ensure that there is a layer of clear buffer above the top of the settled bed. Add additional buffer if required. Assemble the top flow adapter venting air out of the inlet tube.
8. To ensure the column bed is fully packed and stable, it is recommended that the column be run at a minimum flow velocity of 1,000 cm/hr for a minimum of 30 minutes.
9. For Millipore Vantage® L columns, adjust the volume of the ProSep-A media as follows:

A. Close the column outlet.

B. Partially release the flow adapter seal so that the seal pressure is reduced but liquid sealing is maintained.

C. Unlock the flow adapter and pump buffer into the column through the inlet. This will raise the top flow adapter above the bed surface. When the flow adapter is sufficiently clear of the bed, the flow can be stopped, the seal completely released and the flow adapter removed from the column.

D. Excess buffer can be drained off and additional media added to the column as per steps 3-6 above. Run the column at maximum flow rate for at least 30 minutes.

Packing larger scale columns
These are general recommendations for packing larger scale columns. Consult the manufacturer’s recommendations for specific columns.

1. Level the column and half fill the column with packing buffer.

2. Remove any fines by re-suspending the media and allowing to gravity settle for 15 minutes. Decant any fines. Once “de-fined.” pour the media into the column (typically a 50-60% slurry).

3. Insert the top flow adapter leaving as much headspace as possible. Flow upward at 50 cm/hr (bottom of column to top) for two column volumes to expand the ProSep-A media. Do not allow the ProSep-A media to contact the top bed support.

4. Reverse the flow and flow downwards at 100 cm/hr for 2 minutes. Increase the flow to 120% of the maximum process flow rate to pack the bed. When the bed has settled, stop the flow.

5. For intermediate diameter columns (≤ 630 mm diameter), tap the bottom column flange with a rubber mallet to ensure the bed is fully settled. Tapping is not required for larger diameter columns where wall support effects are less.

6. Lower the top flow adapter until it contacts the settled bed and then fix in place.

7. Flow downward repeating steps 4-6 until there is no further settling of the bed. The column is now ready for use.
Adsorption

Since each antibody has different adsorption and elution kinetics, it is preferable to optimize the conditions using a small scale sample prior to large scale processing. Affinity adsorbent dynamic capacity will reduce with increasing flow rate, but productivity will increase. The selection of flow rate depends on the requirements of each individual process. ProSep-A media is typically used between 400 – 800 cm/hr (residence time of 2.5 – 4 min or 20 - 40 column volumes/hr). Higher flow rates can also be used, especially for cleaning and regeneration steps, to reduce cycle time and overall process duration.

1. Before applying the feedstock to the column, wash the column with a minimum of 5 column volumes of the elution buffer, followed by a minimum of 5 column volumes of the binding buffer.

2. Load the column with the feedstock containing the antibody using a pump set at the predetermined optimal flow rate.

3. For optimum binding the following buffers are recommended:
   a) Monoclonal Antibodies
      1. PBS (phosphate buffered saline) pH 7.4
      2. 50 mM phosphate and 0.15 M NaCl pH 7.5
      3. 50 mM phosphate, 0.5M glycine, 0.15M NaCl pH 7.5
      4. 50 mM Tris HCl, 0.15M NaCl, pH 7.5
      5. 1 M glycine/NaOH + 0.15M NaCl pH 8.6
      6. 0.1M borate + 0.15M NaCl pH 8.5
   b) Polyclonal Antibodies
      1. Mouse and Rat:
         1M Glycine + 0.15M NaCl pH 8.6 or 0.1M borate + 0.15 NaCl pH 8.5
      2. Human and other species: PBS pH 7.4

NOTE: Binding buffer greater than pH 8.6 should not be used.
Wash

The wash step removes unbound or weakly bound material from the column.

Wash buffers usually have the same buffer components as the loading buffer to ensure the captured product remains bound to the column while non-binding contaminants are washed off. As the ligand/ligate binding is a reversible equilibrium process, large volumes (>20 column volumes) of wash buffers should be avoided as this may decrease the dynamic capacity of the separation, especially for weakly binding antibodies such as murine MAbs. No more than 10-15 column volumes of wash buffer should normally be required to wash a ProSep-A media, packed bed column.

ProSep-A media may exhibit non-specific binding due to certain process conditions. Such non-specific binding is characterized as the binding of any non-immunoglobulin species present in the feed which then co-elutes with the IgG. In general, the levels of non-specific binding to ProSep-A media are very low and the purity of the eluate can routinely exceed 98%.

In cases where non-specific binding is observed, it is generally due to either ionic or hydrophobic interaction with the base matrix or the immobilized ligand coupling.

In the case of non-specific binding due to ionic interactions, adding salt (1M NaCl) in the post-load wash has been found to be effective.

If the non-specific binding is not addressed by a high salt wash, it may be due to hydrophobic interaction, and lowering the ionic strength of the wash buffer or alternatively adding detergent in the post load wash, for instance 0.1-1.0% Tween® or Triton® X-100, may be effective.

A combination of salt and detergent can be effective if the non-specific binding is due to a combination of ionic and hydrophobic interactions.

Reducing the pH of the wash buffer may also help to remove other contaminants.
**Elution**

To preserve biological activity and reduce IgG denaturation, extremes in pH should be avoided during elution.

All of the product should elute in 3 column volumes or less. If not, check the elution conditions and verify that the molarity of the elution buffer is not less than 0.1M.

The variables to consider in optimizing the elution buffer are pH, buffer components, flow rate and stability of antibody product in chosen buffer. Use the highest possible pH for elution to minimize any risk of denaturation.

The following elution buffers have been found to be optimal for different antibodies.

**Monoclonal Antibodies**

For monoclonal antibodies (i.e., all one subclass), a step elution is usually sufficient. However, the pH required can be determined by preliminary experiments with gradients. The highest pH which will give acceptable recovery of the target antibody should be used. For human IgG this is usually in the region of pH 3.0 - 4.0.

Recommended buffers are 0.1M acetate, citrate or glycine pH 6.0 - 3.0.

**Polyclonal Antibodies**

All subclasses of IgG from different species, especially human IgG can be purified using a pH gradient from pH 6.0 - 3.0 to separate each subclass.

Recommended buffers are 0.1M glycine/HCl

1. Porcine: Elution pH 3.5
2. Human & Other Species: Elution pH 3.0

Following elution and any low pH hold steps for virus inactivation, immunoglobulin solution should be neutralized to pH 6.8 - 7.4 to reduce the possibility of denaturation due to low pH.

The stability of antibody product in the elution buffer should be determined by leaving the antibody in elution buffer for hours or even days and monitoring for any loss in activity. The data will enable the user to estimate the rate of inactivation of antibody in the chosen elution conditions. If there is any inactivation, then the elution buffer should be adjusted immediately after elution. Adjust the pH gradually to minimize the risk of precipitation and denaturation.

Addition of salt can stabilize antibodies if precipitation is observed on adjusting the pH, however this can interfere with subsequent purification steps and should be avoided if possible.
Column Regeneration

After use, regenerate the column with 5 to 10 column volumes of H3PO4 pH 1.5 or HCl pH 1.5, followed by 5 column volumes of PBS or binding buffer.
Cleaning

Cleaning is the removal of residual protein and contaminants from the column. The use of a low pH (pH 1.5) regeneration with phosphoric or hydrochloric acid solution after every cycle is very effective at removing strongly bound material from ProSep-A media. Both protein A and the controlled pore glass base matrix are stable to prolonged exposure at low pH. In contrast, both protein A and the glass base matrix exhibit limited stability at high pH. Where acid alone is insufficient and a stronger cleaning regime is required, a periodic treatment with 2 – 6 M guanidine hydrochloride is recommended (5 column volumes). Frequency of treatment depends on the degree of fouling but typically every 5-10 cycles is found to be effective. If discoloration of the media due to very dirty feeds is experienced, the use of ethanolic acetic acid has been found to be useful. A wash beginning with 20% ethanol/0.5M acetic acid is recommended. It is possible to use higher concentrations of ethanol if necessary but check with local Health & Safety requirements before doing so.

Other useful cleaning solutions include:

- 1-3% Tween or Triton X-100
- 20% ethanol
- 20% ethanol/ 2 M acetic acid
- 0.1M imidazole/20% ethanol, pH 7.5
- Other detergents may be effective, including:
  - 1% Sodium Dodecyl Sulfate,
  - 3% Sodium Deoxycholic Acid
  - 2% Octyl-B-D Glucopyranoside

Do Not Clean With NaOH.
Sanitization

Sanitization is the reduction of bioburden (i.e., micro-organisms and spores) in the column. All columns should be sanitized on a regular basis, such as following each campaign. For ProSep-A media, following normal elution and regeneration using either phosphoric acid or HCl, pH 1.5, wash the column with 5 column volumes of 6M guanidine HCl in water, adjusting the flow rate to ensure a minimum contact time of one hour. The column should then be washed and stored in sterile filtered PBS or binding buffer containing a suitable preservative (e.g. 1% benzyl alcohol).

Contact times with the guanidine hydrochloride, phosphoric acid and HCl, pH 1.5, can be increased up to 8 hours each. This will ensure sanitization and is advised prior to long term storage of the column or if contamination of the column is suspected.

Always use high purity grade guanidine hydrochloride, which should dissolve easily with no trace of insoluble material. Do not heat solutions of guanidine hydrochloride, and do not store prior to use. Always make up fresh solutions as required.
Storage and Handling
ProSep-A media are supplied in 0.1M acetate buffer pH 5.2±0.5 containing 1% benzyl alcohol as a preservative.

Prolonged exposure of this solution to certain materials such as nylon and silicone can affect both the efficacy of the preservative and the physical nature of the material itself.

During use, ProSep-A media may be stored in PBS or other suitable buffer containing a preservative. Store between 2 - 8 °C. DO NOT FREEZE.

If used under the recommended conditions, the product will be reusable over many cycles without significant loss of activity.
## Ordering Information

<table>
<thead>
<tr>
<th>Quantity</th>
<th>ProSep-vA Ultra media</th>
<th>ProSep-vA High Capacity media</th>
<th>ProSep-A High Capacity media</th>
<th>ProSep-rA High Capacity media</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mL pre-packed column</td>
<td>N/A</td>
<td>N/A</td>
<td>113111522</td>
<td>113112522</td>
</tr>
<tr>
<td>2 mL</td>
<td>115115822</td>
<td>113115822</td>
<td>113111822</td>
<td>11311722</td>
</tr>
<tr>
<td>10 mL</td>
<td>115115824</td>
<td>113115824</td>
<td>113111824</td>
<td>11311724</td>
</tr>
<tr>
<td>50 mL</td>
<td>115115826</td>
<td>113115826</td>
<td>113111826</td>
<td>11311726</td>
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<td>115115827</td>
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<td>115115830</td>
<td>113115830</td>
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<td>115115833</td>
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<td>10 L</td>
<td>115115835</td>
<td>113115835</td>
<td>113111835</td>
<td>11311735</td>
</tr>
</tbody>
</table>
**Appendix A Short Form Process Development Protocol**

Recommended Column size: 6.6 mm x 100 mm (3.4 ml)

<table>
<thead>
<tr>
<th>Step</th>
<th>Buffer</th>
<th>Column Volumes</th>
<th>Flow Rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equilibrate</td>
<td>PBS pH 7.4</td>
<td>10</td>
<td>2.0 ml/min</td>
</tr>
<tr>
<td>Load</td>
<td>Direct clarified feed</td>
<td>Dependent on sample volume &amp; concentration</td>
<td>1.3 ml/min (residence time 2.5 min)</td>
</tr>
<tr>
<td>Wash</td>
<td>PBS pH 7.4</td>
<td>10-15</td>
<td>2.0 ml/min</td>
</tr>
<tr>
<td>Elute</td>
<td>0.1M citrate pH 3.5</td>
<td>5</td>
<td>2.0 ml/min</td>
</tr>
<tr>
<td>Regenerate</td>
<td>Phosphoric acid pH 1.5</td>
<td>5</td>
<td>2.0 ml/min</td>
</tr>
<tr>
<td>Re-equilibrate</td>
<td>PBS pH 7.4</td>
<td>10</td>
<td>2.0 ml/min</td>
</tr>
<tr>
<td>Cleaning every 5-10 cycles</td>
<td>6 M guanidine hydrochloride</td>
<td>5</td>
<td>2.0ml/min</td>
</tr>
</tbody>
</table>

*based on 6.6 mm x 100 column

To increase binding capacity
- a) increase pH (maximum pH 9.0)
- b) increase salt concentration to 3M NaCl

Elution
- ascertain elution by running linear gradient from binding buffer to pH 3.0 over 10 column volumes

Non-specific binding
- a) include 1 M NaCl in post-load wash
- b) include 0.1-1% Tween or Triton X-100 in post load wash

*Note:* If feed sample volumes are severely limited, a smaller column (e.g. 2 ml 6.6 mm x 60 mm) may be used for initial binding and elution studies however a minimum 100 mm long column is recommended for dynamic binding determination to ensure reliable data.
Appendix B Buffer Formulation

All formulations are given for the preparation of one liter of buffer

Phosphate Buffered Saline (PBS) pH 7.4
- Disodium hydrogen orthophosphate 3.0g
- Potassium di-hydrogen phosphate 0.2g
- Potassium chloride 0.2g
- Sodium chloride 8.0g
- Distilled water to 1L

or any equivalent PBS formulation

1M Glycine / 0.3M NaCl pH 8.6
- Glycine 75.0g
- Sodium chloride 17.6g
- Distilled water to 1L
  Add Base (NaOH) to pH 8.6

1M Glycine / 0.15M NaCl pH 8.6
- Glycine 75.0g
- Sodium chloride 8.8g
- Distilled water to 1L
  Add Base (NaOH) to pH 8.6

0.1M Borate / 0.15M NaCl pH 8.5
- Boric acid 6.2g
- Sodium chloride 8.8g
- Distilled water to 1L
  Add Base (NaOH) to pH 8.5

0.1M Citrate pH 3 - 6
- Citric acid monohydrate 21.0g
- Distilled water to 1L
  Add Base (NaOH) to required pH

0.1M Glycine / HCl pH 3 – 3.5
- Glycine 7.5g
- Distilled water to 1L
  Conc. Hydrochloric acid to required pH

10mM HCl pH 1.5
- Conc. Hydrochloric acid 3.0 mL
- Distilled water to 1L

0.25M Phosphoric acid pH 1.5
- Conc. Phosphoric acid 10.0 mL
- Distilled water to 1L

6M Guanidine hydrochloride
- Guanidine hydrochloride 573.2g
- Distilled water to 1L
Bibliography


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