Data sheet

Super Q Plus IEX Resin

Super DEAE Plus IEX Resin

Super SP IEX Resin
Super CM Plus IEX Resin

1. Introduction

Super Q Plus, DEAE Plus, S Plus and Super CM Plus are a group of strong anion exchange, weak anion exchange, strong cation exchange and weak cation exchange media with tentacle chains, respectively. The binding capacity and binding kinetics is much higher than our Super IEX resins (Q, DEAE, SP & CM). Neo Bitoech's Super Plus range of resins is particularly useful, due to its high resolution with high loading capacity, in a process that requires to purify difficult molecules at high throughput and cost-effective ways.

Neo Biotech's DEAE & CM Super Plus media has a balanced design to offer high resolution power and also high loading capacity to separate similar components in bioprocessing applications. The core advantages are:

- High sample loading capacity at high flow
- High resolution at high loading

The base matrix is made of highly cross-linked agarose and dextran with excellent flow property. It is very stable to most of the chemical conditions experienced in the bioprocessing industry.

2. Product properties

Characteristics of the ion-exchange Super Plus Resin Media:

	Super Q Plus	Super DEAE Plus	Super SP Plus	Super CM Plus
Matrix	Highly cross-linked agarose grafted with dextran chain			
Functional group	Quaternary ammonium strong anion	Diethylaminoethyl weak anion	Sulfopropyl strong cation	Carboxymethyl weak cation
Total ionic capacity	0.09-0.15 mmol/ml	0.08-0.13 mmol/ml	0.09-0.15 mmol/ml	0.08-0.13 mmol/ml
Dynamic binding capacity* / ml	>130 mg BSA / ml	>130 mg BSA / ml	>130 mg lysozyme	>130 mg lysozyme / ml
Particle size	20 - 50 μm			
Pressure-flow property	30 -150 cm/hr is recommended			
Operational pressure	Up to 3 bar			
pH stability	2-14 (short term) and 3-12 (long term)		2-14 (short term) and 4-12 (long term)	
Working temperature	+4°C to +30°C			
Chemical stability	All commonly used buffers; 1 M acetic acid, 1 M NaOH, 6M guanidine hydrochloride, 8 M urea, 30% isopropanol, 70% ethanol			
Avoid	Oxidizing agents, anionic detergents		Oxidizing agents, cationic detergents	
Storage	20% ethanol	20% ethanol	20% ethanol + 0.2M NaAc	20% ethanol

Test condition: 10 mM buffer pH 7.5, 5 mg/ml protein to load, residence time 5 min, 50% breakthrough

3. Method optimization

We recommend scouting the parameters among loading capacity, flow velocity, binding pH, binding ionic strength, elution speed and gradient etc. Due to the fast pore accessibility of our Super Plus media, the binding step could be achieved in a faster flow velocity than that in the elution step. We recommend to pay special attention to optimize elution conditions to achieve the best separation power.

Strong ion exchange media maintain their charges (and thus their function) over a wide pH range whereas with weak ion exchange media the degree of dissociation and thus ion exchange capacity varies with pH. Therefore, it is more critical to optimize the pH if weak ion exchange media is used.

In general, balancing the degree of component separation against process throughput is the major consideration when optimizing a method. Besides, for the purification of instable or shearing-force sensitive molecules, the operational condition needs be optimised to balance the throughput and the possible damage to the target molecule.

4. Column packing

Neo Biotech's Super Plus ion-exchange media is made of highly cross-linked beaded agarose with excellentmechanical strength. The media can be easily packed in any type of chromatography columns at any possible packing mode. The typical packing pressure is up to 3 bars.

Packing large-scale columns – General recommendations
Columns can be packed in different ways depending on the type of column and equipment used. Please refer to the relevant column instruction manual carefully.

Note: As the columns increase in diameter the packing flow rate decreases, at packing flow rates below 150cm/hr there is generally little impact, at higher flow rates a 3 fold increase in column diameter can increased packing pressure approximately 2 fold.

In general there are three suitable types of packing methods:
- Pressure packing (for columns with moveable adaptors).
- Combined pressure/suction packing (for medium sized columns with fixed bed heights).
- Suction packing (for large columns with fixed bed heights).
- Hydraulic pressure packing.

How well the column is packed will have a major effect on the performance of the resin and the purity and yield of the purification process. Guidelines are given for determining the optimal packing flow rates for different column designs columns with specific design features like adaptors and fixed bed heights.

Determining the optimal packing pressure
The optimal packing pressure/flow rate is dependent on column size, type, desired bed height, packing solution and temperature. The optimal packing flow rate must therefore be determined empirically for each individual system. Generically this is done as follows:

1. Calculate the exact amount of resin needed for the slurry (this is especially important for columns with fixed bed heights). Extra resin is required to allow for settling of the bed, e.g. allow approximately 1.15L of resin slurry per 1 liter of packed bed.
2. Prepare the column per the column instructions.
3. Begin packing the column at a low flow rate (e.g. 30% of the expected max process flow rate), record the flow rate and back pressure when the bed is completely packed and the pressure has stabilized.

4. Increase flow rate recording both flow rate and pressure drop in a stepwise manner always allowing the pressure to stabilize at each step.

5. Continue recording flow and pressure until the maximum process flow rate has been reached. This reached when the pressure flow curve levels off or the maximum column pressure is reached.

6. Plot pressure against flow rate.

The optimal packing pressure is about 70% of the maximum pressure. From the packing pressure point on the curve, draw a straight line to zero. The maximum operational pressure should be <70% of the packing pressure. From the straight line, the maximum operational flow rate can be found.

Pressure Packing – typically for columns supplied with a movable top flow plate (e.g., GE BPG™; Millipore Vantage™ and Quicksale™) are packed by conventional pressure packing where packing solution is pumped through the settling chromatographic bed at a constant back pressure. Specific packing instructions and pressure flow curves are generally provided by the column manufacturers and can be matched with each resins pressure flow properties to develop a robust packing protocol for each column/resin combination. Generically the steps are as follows:

1. Make sure no air is trapped under the bottom bed support by pumping packing buffer through it from below. Excess liquid in the column can be removed by connecting tubing to the suction side of a pump. Leave about 2 cm of liquid in the column.

2. Mix the packing buffer with the medium to form a 50% slurry (settled bed volume/slurry volume = 0.5). Pour the slurry into the column. Insert top distributor plate the adaptor and lower to the surface of the slurry, making sure no air is trapped under the plate and secure in place.

3. Fill the adaptor inlet with packing solution.

4. Connect a pump and a pressure meter; apply a flow that gives the proscribed back pressure (typically about 0.1 bar). When the bed has settled, run for a few minutes, close the valve and stop the pump. Lower the plate down to the top of the bed.

5. Start the pump and apply a flow that gives the desired packing pressure. Keep the pressure constant during packing and check the pressure at the column inlet. **Never** exceed the pressure limit for column or medium. Run for at least 15 min.

6. When the bed has stabilized, mark the bed height, close the valve and stop the pump.

7. Disconnect the column inlet tubing and replace it with tubing leading to waste, push the top plate adaptor down to approximately 3 mm below the mark on the column tube. The packing solution will flush the adaptor inlet. Remove any trapped air by pumping liquid from the bottom (after the inlet tubing and the bottom valve have been properly filled).

Combined pressure/suction packing- typically these columns have a fixed bed height of 15 cm. It is packed by a combined pressure/suction technique. Follow the column manufacturer's instructions, which generically include:

1. Fitting an extra column section on top of the column tube as a packing reservoir.

2. Pour water or packing buffer into the column making sure that there is no air trapped under the bottom bed support. Leave about 2 cm of liquid in the column.

3. Pour the slurry into the column. Stir gently to give an homogeneous slurry. Add buffer until level with the upper rim and secure the lid in place.

4. Connect a pump and a pressure meter and start packing at the predetermined packing flow rate (or pressure). Keep the flow rate (or pressure) constant during packing and check the pressure at the column inlet. Never exceed the pressure limit for the column or medium.

5.   When the bed has stabilized, the top of the bed should be exactly level with the top of the column tube. Switching the valve takes the buffer tank off line the inlet pump is now connected to the outflow side of the column. The packing buffer is re-circulated in the system. If, when stabilized, the packed bed is not exactly level with the top of the column, add or remove slurry.

6.   Keep the pump running, disconnect the column inlet and direct it to waste. The packing solution in the packing section is removed by suction through the bed.

7.   Remove the packing reservoir section.

8.   When the packing solution is within 5-8 mm of the bed surface stop the pump. This final operation should be completed as quickly to prevent bed expansion.

9.   Start pumping buffer with upward flow through the column to remove any air bubbles.

Suction Packing – typically for columns with fixed end pieces. These columns are packed by suction, i.e. by sucking packing solution through the chromatographic bed at a constant flow rate.

1.   Fit a packing device on top of the column tube.

2.   Pour water or packing buffer into the column making sure that there is no air trapped under the bottom bed support. Leave about 2 cm of liquid in the column.

3.   Mix the packing buffer with the medium to form a 50% slurry (settled bed volume/slurry = 0.5). Pour the slurry into the column.

4.   Connect the column outlet valve to the suction side of a pump and start packing the bed by suction through the bed at the predetermined flow rate. Keep the flow rate constant during packing.

5.   When the bed has stabilized, the top of the bed should be just below the junction between the column and the packing device.

6.   Just before the last of the packing solution enters the packed bed, stop the pump and quickly remove the packing device and replace it with the lid. This final operation should be completed quickly to prevent bed expansion when the flow stops.

7.   Start pumping buffer with upward flow through the column to remove any air bubbles trapped under the lid.

Hydraulic Packing- is for columns supplied with a hydraulic function GE INdEX™ and FineLine™; Novasep Prochrom® DAC. In these systems and automated hydraulic systems controls packing as the adaptor is lowered into position at the correct pressure. The adaptor is pushed down by a constant hydraulic pressure, forcing packing buffer through the slurry and compressing it so that a packed bed is gradually built up.

The quantity of medium required when packing our resin by hydraulic pressure is approximately 1.15L of resin slurry per 1 liter of packed bed. Generically packing is completed as follows:

1.   Make sure that there is no air trapped under the bottom bed support, by pumping packing buffer through it from below. Leave about 2 cm of liquid in the column.

2.   Pour the slurry into the column. Fill the column with packing solution up to the top of the tube allowing the medium bed to settle just below the top of the column tube.

3.   Put the adaptor in a resting position in the column tube and lower the lid and secure it in place.

4.   Connect a pump to the inlet, to start the packing, applying a predefined constant hydraulic packing pressure. When packing IPA 400HC in this type of column pack the bed to less than the recommended operational pressure.

5. When the adaptor has reached the surface of the settled bed, continue to run the pump until the adaptor has been lowered fractionally into the packed bed (depending on the column manufacturer's instructions)

Packing Efficiency Assessment

To check the quality of the packing and to monitor this during the working life of the column, column efficiency should be tested directly after packing, prior to re-use and if there is an observed deterioration in separation performance. The efficiency of a packed column is expressed in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (As). These values are easily determined by applying a sample such as 1% acetone solution to the column and using water as eluent. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent. It is important that conditions and equipment are kept constant so that results are comparable.

Changes in solute, solvent, eluent, sample volume, flow rate, liquid pathway, temperature, etc., will influence the results. A sample volume of less than 2.5% of the column volume and the flow velocity between 15 and 30 cm/h will give the most optimal results.

Method for measuring HETP and Asymmetry

Important: For best results avoid sample dilution by applying it as close to the column inlet as possible, and placing the UV meter as close to the column outlet as possible.

Conditions
- Sample volume: 1.0% of the bed volume
- Sample conc.: 1.0% v/v acetone
- Flow velocity: 20 cm/h
- UV: 280 nm, 1 cm, 0.1 AU

Calculate HETP and A_s from the UV curve (or conductivity curve if NaCl is used as sample) as follows: HETP = L/N

$$N = 5.54 \ (Ve \ / \ Wh)^2$$

Where L = Bed Height (cm)

N = Number of theoretical plates Ve

= Peak elution distance

Wh = Peak width at half peak height Ve

and Wh are in the same units.

To facilitate comparison of column performance the concept of reduced plate height is often used. *Reduced plate height is calculated: HETP/d where d is the mean diameter of the bead. As a guideline, a value of < 3 is normally acceptable.*

For a well-packed efficient column the peak should be symmetrical, and the asymmetry factor as close as possible to 1 (values between 0.8-1.5 are usually acceptable). A change in the shape of the peak is usually the first indication of bed deterioration due to use.
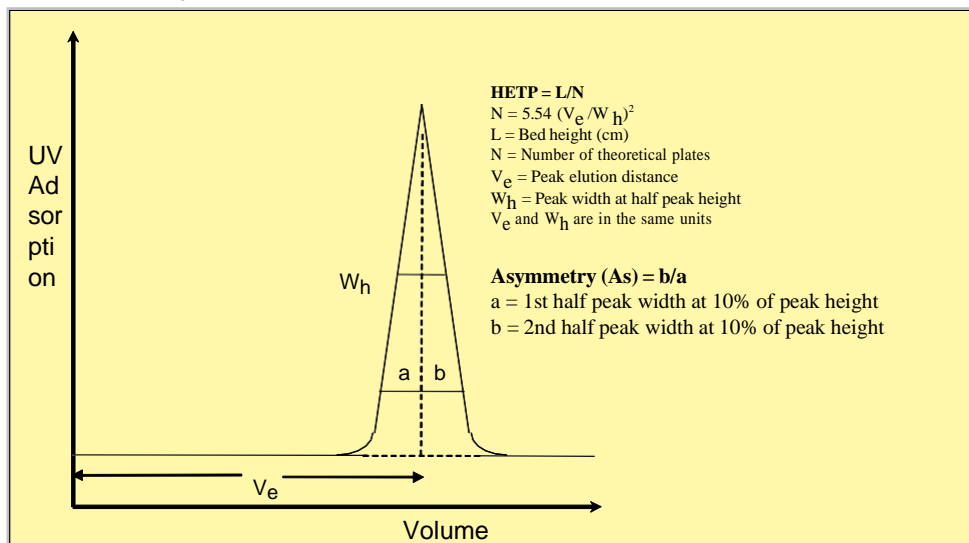
Peak asymmetry factor calculation:

A_s = b/a where

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height

Figure 1 shows a UV trace for acetone in a typical test chromatogram in which the HETP and As values are calculated.

Figure 1. HETP and A_s Calculations.

In the figure:

HETP = L/N
$N = 5.54 (V_e / W_h)^2$
L = Bed height (cm)
N = Number of theoretical plates
V_e = Peak elution distance
W_h = Peak width at half peak height
V_e and W_h are in the same units

Asymmetry (As) = b/a
a = 1st half peak width at 10% of peak height
b = 2nd half peak width at 10% of peak height

If the column is packed according to the instructions described above typical values obtained for Protein A SepFast resin should be:

Number of theoretical plates > 3,000 Peak asymmetry 0.8-1.5

5. Maintenance

Depending on the individual applications, the media may be used many times. For the re-use purpose, please see the following instructions.

Regeneration
After each run, elute any reversibly bound material either with a high ionic strength solution (e.g. 1M NaCl in buffer) or by increased pH.

Cleaning-in-place (CIP)
CIP is a procedure that removes strongly bound materials such as lipids, endotoxins and denatured proteins that remain in the adsorbent surface after regeneration. Regular CIP prevents the build up of contaminants in the packed bed and helps to maintain the column performance.

A specific CIP protocol should be developed for each process according to the type of contaminants present. The frequency of CIP depends on the nature of individual applications.

The following information works as a general guidance.

Salt with concentration up to 2 M can be used to clean the impurities bound by ionic interactions. The contaminants bound by hydrophobic nature can be removed by the following reagents: 1 M NaOH, low percentage non-ionic detergents (e.g. 0.1 – 2%), 30% isopropanol in basic or acidic conditions (e.g. in the presence of acetic acid or phosphoric acid). A combination of the above reagents can be explored as well. In general, the incubation time should be longer (e.g. from 30 minutes to 2 hours) to ensure full dissociation of the contaminants.

Sanitization
Sanitization using 0.5-1.0 M NaOH with a contact time of 1 hour is recommended.

6. Storage

The media should be stored in 20% ethanol (or 20% Ethanol plus 0.2M NaAc for the SP resin) to prevent microbial growth. Store the media at a temperature of +4°C to +30°C. Before use, equilibrate the media with at least 5 bed volumes of the running buffer.

Ordering Information

Product Description	Pack Size	Product code
Super Plus CM Resin	25ml 100ml 500ml 1L 5L 10L	NB-45-0225-25 NB-45-0225-100 NB-45-0225-500 NB-45-0225-1L NB-45-0225-5L NB-45-0225-10L
Super Plus DEAE Resin	25ml 100ml 500ml 1L 5L 10L	NB-45-0226-25 NB-45-0226-100 NB-45-0226-500 NB-45-0226-1L NB-45-0226-5L NB-45-0226-10L
Super Plus Q Resin	25ml 100ml 500ml 1L 5L 10L	NB-45-0227-25 NB-45-0227-100 NB-45-0227-500 NB-45-0227-1L NB-45-0227-5L NB-45-0227-10L

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