USER GUIDE: Ion Exchange resin

This material is for the following products

EBL IEX_Cation **CM**, IEX_Cation **S**, IEX_Cation **SP**, IEX_Anion **DEAE**, and IEX_Anion **Q**

Introduction:

CM, S, SP, DEAE, and Q are ion exchange chromatography media with excellent flow properties, high performance and high capacity for biomolecules. The instructions that follow are based upon packing EBL ion exchange resin. To modify these instructions for columns of different dimensions, refer to "Appendix A". EBL resin can also be packed in other columns. Read and follow the column instruction manual. Detailed information on the technique of ion exchange can be found in numerous other sources.

Recommended Procedure and Working Conditions:

Preparing the gel

1. Equilibrate all material to room temperature.
2. Decant the shipping solution and replace it with packing buffer to a total volume of 32.5 ml (75% settled gel: 25% buffer). Note: EBL ion exchangers CM, DEAE and Q are supplied in suspension in 20% ethanol as standard shipping solution. SP is supplied in 20% ethanol and 0.2 M sodium acetate as standard.
3. Degas the slurry under vacuum

Assembling the column

Details of the column parts can be found in the instructions supplied with the column.

1. Before packing ensure that all parts, particularly the nets, net fasteners and glass tube, are clean and intact.
2. Connect the column bottom end piece to a pump or syringe.
3. Submerge the end piece in buffer and fill it using the pump or syringe. Ensure that there are no air bubbles trapped under the net.
4. Close the tubing with a stopper and mount the end piece on the column.
5. Flush the column with buffer, leaving a few ml at the bottom. Mount the column vertically on a laboratory stand.

Packing the column

The instructions that follow are for packing EBL ion exchange resin in the recommended 16/20 Column. To modify these instructions for columns of different dimensions, refer to "Appendix A".

1. Pour the gel slurry into the column in one continuous motion. Pouring down a glass rod held against the wall of the column helps prevent the introduction of air bubbles. Fill the remainder of the column with buffer.
2. Wet the column adaptor by submerging the plunger end in buffer, and drawing buffer through with a syringe or pump. Ensure that all bubbles have been removed.
3. Disconnect the pump or syringe.
4. Insert the adaptor into the top of the column at an angle, taking care not to trap air under the net. Tighten the adaptor O-ring to give a sliding seal on the column wall.
5. Fit a syringe barrel to the sample application valve and connect the valve between the adaptor and the pump.
6. With the valve in the sample application position, slide the adaptor down into the column. This will displace all air in the tubing as far as the sample application valve. Switch the valve a few times to remove any trapped bubbles.
7. Continue inserting the adaptor until it reaches the gel slurry. Tighten the O-ring and lock the adaptor in position.
8. Open the bottom outlet of the column and start the pump. Pack the gel at a flow rate of 12 to 14 ml/min. Pack at this flow rate until the bed height is constant (normally 4 to 5 minutes). Note: Ideally, EBL resins are packed at a constant pressure of 0.2 MPa (2 bar) for a 16 mm diameter column.
9. Stop the pump, close the column outlet, loosen the adaptor O-ring to give a sliding seal and re-position the adaptor on the surface of the gel bed.
10. Press the adaptor into the surface of the gel an additional 1 to 2 mm. Lock the adaptor in position, open the column outlet and start the pump at the column packing flow rate. If the bed continues to pack, repeat step 5. When the gel bed is stable, the column is packed equilibrated and ready for use. If required, the quality of packing can be checked using the testing procedure described in “Appendix B”.

Equilibration

To equilibrate, pump approximately 100 ml of start buffer through the column at a flow rate of 8 to 10 ml/min. The column is fully equilibrated when the pH and/or conductivity of the effluent is the same as the start buffer.

Sample Preparation

The amount of sample that can be applied to the column depends on the available capacity of the ion exchanger and the degree of resolution required. For best resolution it is usually not advisable to exceed 10 to 20% of the available capacity. See Table 1 in “Appendix C” for capacity guidelines. The sample should be dissolved in start buffer. Alternatively, the sample may be transferred to start buffer by dialysis or by buffer exchange using a Desalting or a Gel filtration column. The viscosity of the sample should not exceed that of the buffer. For

normal aqueous buffer systems, this corresponds to a protein concentration of approximately 50 mg/ ml. Before application the sample should be centrifuged or filtered through a 0.45 µm filter to remove any particulate matter.

Operating flow rates

The flow rate used for sample binding and subsequent elution will depend on the degree of resolution required, but is normally within the range 10 to 15 ml/min (300 to 450 cm/h). The lower the flow rate, the better the resolution.

Binding

The most common procedure in ion exchange is to bind the molecules of interest, while allowing contaminants to pass through the column. In some cases, however, it may be more useful to bind the contaminants and allow the molecules of interest to pass. For efficient binding, the ionic strength of the starting buffer must be low. The pH should be at least one pH unit different from the isoelectric point (pI) of the molecules to be bound and within 0.5 pH units of the selected buffer salt's pKa.

Note: For DEAE and Q the starting buffer must be at least one pH unit above the pI of the molecule to be bound. For CM and SP the starting buffer must be at least one pH unit below the pI of the molecule to be bound. Recommended buffers for different pH´s can be found in "Appendix C", Table 2 and Table 3

Elution

A linear gradient of increasing sodium chloride concentration is the most commonly used elution method in ion exchange. A suggested gradient is from start buffer containing no NaCl to start buffer containing 0.5 M NaCl over a volume of 400 ml. Increase the salt concentration if the substance of interest is not eluted in the gradient. Linear pH gradients and stepwise salt concentration and pH gradients may be useful in some circumstances.

Regeneration

Regeneration is normally performed by washing with a high ionic strength buffer (e.g., start buffer containing 1 to 2 M NaCl) and/or changing pH, followed by re-equilibrating in starting buffer. Regeneration can be carried out at flow rates of 8 to 10 ml/min. Monitor the UV absorbance during regeneration to determine when bound substances have been completely washed out of the column. In some applications, substances such as denatured proteins or lipids do not elute in the regeneration procedure. These can be removed by cleaning-in-place procedures.

Cleaning-in-place (CIP)

- Remove ionically bound proteins by washing the column at 1 to 1.5 ml/min in a reversed flow direction with 10 ml of a 2 M NaCl solution, contact time 10 to 15 minutes.
- Remove precipitated proteins, hydrophobically bound proteins and lipoproteins by washing the column in a reversed flow direction with 100 ml 1 M NaOH solution at a flow rate of 1.2 to 1.4 ml/min.
- Remove strongly hydrophobically bound proteins, lipoproteins and lipids by washing the column in a reversed flow direction with 80 ml of 70% ethanol or 30% isopropanol at a flow rate of 1.2 to 1.4 ml/min.

Apply increasing concentration gradients to avoid air bubble formation, when using high concentrations of organic solvents. After cleaning the column, equilibrate with approximately 100 ml of start buffer before use.

Sanitization

Sanitization reduces microbial contamination of the gel bed to a minimum. Wash the column in the reversed flow direction for 30 to 60 minutes with 0.5 to 1 M NaOH, at a flow rate of 1.2 to 1.4 ml/min. Re-quilibrate the column with approximately 100 ml sterile start buffer.

Storage

Unopened media can be stored at +4°C to +30°C. Used Q, DEAE and CM resin and columns filled with these media should be stored in 20% ethanol. Used SP resin and columns filled with this media should be stored in 20% ethanol and 0.2 M sodium acetate.

Appendix A

Converting to columns of different dimensions

Flow rates

Flow rates quoted in this instruction are for a 16/20 column. To convert flow rates for columns of different dimensions: 1 Divide the volumetric flow rates (ml/min) quoted by a factor of 2 (the cross-sectional area in cm2 of the 16/20) to give the linear flow rate in cm/min. 2 Maintain the same linear flow rate and calculate the new volumetric flow rate according to the cross-sectional area of the specific column to be used

$$\text{Linear flow rate} = \frac{\text{Volumetric flow rate}}{\text{Column cross-sectional area}}$$

Volumes

Volumes (buffers, gradients, etc.) quoted in this instruction are for a 16/20 column that has a bed volume of 20 ml (bed height × cross-sectional area). To convert volumes for columns of different dimensions, increase or decrease in proportion to the new column bed volume.

$$\text{New volume} = \text{Old volume} \times \frac{\text{New bed volume}}{\text{Old bed volume}}$$

Appendix B

Testing the packed column

To check the efficiency of the column packing, determine the theoretical plate number and peak symmetry. If the column is packed according to the instructions described above typical values should be:

Efficiency: N > 3, 000 theoretical plates per meter

Peak symmetry: As = 0.80 - 1.50

Solutions required:

20% (v/v) ethanol in distilled water

Sample: acetone 0.2% (v/v) in 20% ethanol

Test Protocol:

1. Establish a flow rate of 4 ml/min (i.e., a linear flow rate of 120 cm/hour) through the packed 16/20 column with 20% ethanol.
2. Set the monitor at 280 nm at an absorbance of 1.0 AUFS for a 10 mm path length cell. Run the chart recorder at a minimum of 10 cm/min. Zero the monitor and chart recorder.
3. Inject 200 µl acetone (100 µl per cm2 cross-sectional area) onto the column at a flow rate of 4 ml/min (linear flow rate of 120 cm/hour). Record the absorbance from the time of injection until the acetone peak has been detected and the monitor signal has returned to baseline.

   Note: If the chart recorder tracing of the acetone peak is not at least 70% of the full scale chart deflection, increase the sensitivity of the monitor setting accordingly and re-run.
4. Calculate the column efficiency (plate number). Referring to the figure below, calculate the column efficiency (N) as follows:

   $$2N = 5.54\ (V_e / W_{1/2})x\ (1000/L)$$

   Referring to the figure below, calculate the symmetry factor (AS) by the formula: As = b/a UV

Troubleshooting:

- If the efficiency is low (i.e., N < 3,000 plates per meter), repeat column packing step 5, and re-test the column.
- If the peak tails badly (i.e., AS > 1.3), repeat column packing step 5, and re-test the column.
- If the peak fronts badly (i.e., AS < 0.7), empty the gel from the column, re-pack the column, and re-test.

Appendix C

Table 1: IEX resin characteristics:

	CM	**SP**	**S**	**DEAE**	**Q**
Characteristic	weak cation	strong cation	strong cation	Weak anion	strong anion
Ligand	Carboxymethyl	Sulphopropyl	Sulfonate group	Diethylaminoethyl	Quaternary amine
Functional group	-CH2-COO^-	-CH_2-CH_2-CH_2-SO_3^-	-CH_2-SO_3^-	-CH_2-CH_2-N^+-(CH_2-CH_3)$_2$	-CH_2-N^+-(CH_3)$_3$
Bead structure	Highly cross-linked polyacrylate polymer				
Particle size（μm）	50 ± 5 %				
Total ionic capacity	0.09-0.13 mmol H^+/ml medium	0.18-0.25 mmol H^+/ml medium	0.18-0.25 mmol H^+/ml medium	0.11-0.16 mmol (Cl^-)/ml	0.18-0.24 mmol Cl^-/ml medium
Available capacity	IgG, 15 mg/ml Ribonuclease, 50 mg/ml	Ribonuclease, 70 mg /ml	Ribonuclease, 70 mg /ml	Thyroglobulin, 3.1 mg/ml HAS, 110 mg/ml a-lactalbumin, 100 mg/ml	Thyroglobulin, 3 mg/m HAS, 120 mg/ml
Max. linear flow rate	≧1250 cm/hour				
Suggested Flow Rate	150-700（cm/h）				
pH stability (long term/ short term)	4-13/ 2-14	4-13/ 3-14	4-13/ 3-14	3-12/ 1-14	2-12/ 1-14

Chemical stability: All commonly used aqueous buffers. 1.0 M NaOH, 8 M Urea, 8 M guanidine hydrochloride, 70% ethanol (tested at 40°C for 7 days).

Physical stability: Negligible volume variation due to changes in pH or ionic strength.

Autoclavable: With counter-ions (CM & SP Na+; DEAE & Q Cl-) at 121°C, pH 7 for 30 min.

Table 2. Suggested buffers for use with DEAE and Q media:

	Counter ion	Concentration	pKa(25°C)
N-ethylpiperazine	Cl^-	20 mM	4.8
piperazine	Cl-, $HCOO^-$	20 mM	5.7
L-histidine	Cl^-	20 mM	6.0
bis-Tris	Cl^-	20 mM	6.5
bis-Tris propane	Cl^-	20 mM	6.8
triethanolamine	Cl-, CH_3COO-	20 mM	7.8
Tris	Cl^-	20 mM	8.1
N-methyldiethanolamine	SO_4^{2-} and CH_3COO^-	50 mM	8.5
Diethanolamine	Cl^-	20 mM at pH 8.4 50 mM at pH 8.8	8.9
1,3-diaminopropane	Cl-	20 mM	8.6
Ethanolamine	Cl-	20 mM	9.5
Piperazine	Cl-	20 mM	9.7
1,3-diaminopropane	Cl-	20 mM	10.5

Table 3. Suggested buffers for use with CM, S and SP media:

	Counter ion	Concentration	pKa (25°C)
Citrate	Na^+, Li^+	20 mM	3.1
Acetate	Na^+, Li^+	50 mM	4.8
Malonate	Na^+, Li^+	50 mM	5.7
Phosphate	Na^+	50 mM	7.2
Bicine	Na^+	50 mM	8.4

Ordering information:

CAT NO:	Product	Quantity