Life Sciences

AcroSep™ Columns for Ion Exchange Chromatography

- Pre-packed columns for chromatographic separations.
- Patented HyperD® "gel-in-a-shell" resin offers high dynamic binding capacities at fast flow rates for greater productivity at competitive resolution.
- Versatile use:
  – fully automated – in combination with an automated chromatography instrument such as the ÄKTAdesign* systems
  – semi-automated – in combination with pumps
  – manually – in combination with a syringe

Ordering Information

Prod. No.	Description	Color Code	Column Volume (CV)	Packaging
20050-C001	CM Ceramic HyperD F	Green	1 mL	5/pkg
20062-C001	S Ceramic HyperD F	Sky Blue	1 mL	5/pkg
20066-C001	Q Ceramic HyperD F	Red	1 mL	5/pkg
20067-C001	DEAE Ceramic HyperD F	Orange	1 mL	5/pkg

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Note: *The procedures herein are intended only as a guide. Users should always verify product performance with their specific applications under actual use conditions. If you have questions about the information presented in this guide, please contact Pall Life Sciences technical service.*

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Specifications

Materials of Construction

Column Housing, Cap, Plug and Adapter: Polypropylene
Column Frit: Polyethylene

Ion Exchange Resin

Media	Function	Particle Size	Stability Range pH	Cleaning pH	Ion Exchange Capacity (1)
CM Ceramic HyperD® F	Weak Cation Exchanger	50 µm (avg)	2-12	1-14	> 60 mg/mL (2)
DEAE Ceramic HyperD F	Weak Anion Exchanger	50 µm (avg)	2-12	1-14	> 85 mg/mL (3)
Q Ceramic HyperD F	Strong Anion Exchanger	50 µm (avg)	2-12	1-14	> 85 mg/mL (3)
S Ceramic HyperD F	Strong Cation Exchanger	50 µm (avg)	2-12	1-14	> 75 mg/mL (4)

(1) Dynamic binding capacity determined at 10% breakthrough, 200 cm/h with 7 mL resin packed in a column of 1.1 cm ID and 7 cm height using:

(2) 5 mg/mL human IgG in 50 mM sodium acetate buffer, 100 mM NaCl, pH 4.7

(3) 5 mg/mL BSA in 50 mM Tris-HCl buffer, pH 8.6.

(4) 5 mg/mL lysozyme in 50 mM sodium acetate buffer, pH 4.5

Specifications (continued)

Column Geometry
Column Volume: 1.04 mL
Bed Height: 1.48 cm (0.58 in)
Bed Diameter: 0.94 cm (0.37 in)

Device Dimensions
Diameter: 1.6 cm (0.6 in)
Length (Without Plugs): 4.8 cm (1.9 in)

Connections
Inlet: Threaded female luer
Outlet: Rotating male luer locking hub

Flow Rates
Recommended: 1-4 mL/min

Back Pressure
Maximum: 3 bar (300 kPa, 43.5 psi)

Storage
2-8 ºC

Instructions for Use

1. Determination of Ion Exchanger
   a. A charged molecule will bind to an oppositely charged matrix.
   b. If the pH of the buffer is below the pI of the target molecule, the net charge on the target molecule will be positive and will therefore bind to a cation exchanger (CM Ceramic HyperD® F or S Ceramic HyperD F).
   c. If the pH of the buffer is above the pI of the target molecule, the net charge on the target molecule will be negative and will therefore bind to an anion exchanger (Q Ceramic HyperD F or DEAE Ceramic HyperD F).

2. Selection of Buffers
   a. Do not use water in the preparation, washing, or regeneration of the Ceramic HyperD resins.

Instructions for Use (continued)

b. Equilibration and protein loading buffer
  - To achieve optimal binding capacity using the Ceramic HyperD resins (except CM Ceramic HyperD), we strongly recommend loading the protein in a buffer with **4-6 ms/cm**.
  - To achieve optimal binding capacity and performance using the CM Ceramic HyperD, load in a buffer concentration of **75-100 mM**.
  - The ion exchange HyperD resins have a higher tolerance for salt than most ion exchange resins. In particular, CM HyperD can be used with up to 100 mM NaCl with only moderate decreases in capacity.

c. Elution buffer
  - The elution and loading buffers are usually the same with elution buffer containing salt, generally NaCl up to 1.0 M.
  - Ceramic HyperD® resins can be used with any typical elution protocol, although slightly higher concentrations of salt may be required (as compared to other ion exchange resins) to achieve the same degree of elution.

d. Buffer pH
  - The initial pH of any buffer for cation exchange should be at least 1 pH unit below the pI of the target molecule. Note that pI is usually calculated based on amino acid sequence. Actual pI after post-translation modifications might differ.
  - The initial pH of any buffer for anion exchange should be at least 1 pH unit above the pI of the target molecule.
  - If pH based elution is used, ensure that no solubility problems will occur during the pH transition. Salt can be used to help maintain solubility during pH changes.

Instructions for Use (continued)

Buffers for Cation Exchange Chromatography

For optimal binding capacity using the S Ceramic HyperD® resin use a buffer with 4-6 ms/cm conductivity.

To achieve optimal binding capacity and performance using the CM Ceramic HyperD, use a buffer concentration of 75-100 mM.

pH interval	Substance	pKa (25 °C)
1.4-2.4	Maleic acid	1.92
2.6-3.6	Methyl malonic acid	3.07
2.6-3.6	Citric acid	3.13
3.3-4.3	Lactic acid	3.86
3.3-4.3	Formic acid	3.75
3.7-4.7; 5.1-6.1	Succinic acid	4.21; 5.64
4.3-5.3	Acetic acid	4.75
5.2-6.2	Methyl malonic acid	5.76
5.6-6.6	MES	6.27
6.7-7.7	Phosphate	7.20
7.0-8.0	HEPES	7.56
7.8-8.8	BICINE	8.33

Ref: Handbook of Chemistry and Physics, 87th edition, CRC, 2006-2007

Instructions for Use (continued)

Buffers for Anion Exchange Chromatography

For optimal binding capacity with the anion exchange Ceramic HyperD® resins use a buffer with 4-6 ms/cm conductivity.

pH interval	Substance	pKa (25 °C)
4.3-5.3	N-Methylpiperazine	4.75
4.8-5.8	Piperazine	5.33
5.5-6.5	L-Histidine	6.04
6.0-7.0	bis-Tris	6.48
6.2-7.2; 8.6-9.6	bis-Tris propane	6.65; 9.10
7.3-8.3	Triethanolamine	7.76
7.6-8.6	Tris	8.07
8.0-9.0	N-Methyl- diethanolamine	8.52
8.0-9.0	N-Methyl- diethanolamine	8.52
8.4-9.4	Diethanolamine	8.88
8.4-9.4	Propane 1,3-Diamino	8.88
9.0-10.0	Ethanolamine	9.50
9.2-10.2	Piperazine	9.73
10.0-11.0	Propane 1,3-Diamino	10.55
10.6-11.6	Piperidine	11.12

Ref: Handbook of Chemistry and Physics, 87th edition, CRC, 2006–2007

3. Reagent Preparation – Particulate Removal
   a. Buffer preparation
      – Filter through 0.2 µm filter after buffer preparation and pH adjustment.
   b. Sample preparation
      – Filter through 0.2 µm filter immediately before use.

Instructions for Use – Automated or Pumped Chromatography Systems

1. Purification
   a. Attach column to pre-primed system (ÄKTAdesign*, pump, or equivalent) and while system is running very slowly, fill the neck of the column dropwise to prevent air from getting into the column. Allow the buffer to flow all the way through the column until all bubbles in the bottom of the column have been evacuated.
   b. Wash column with 5 column volumes (CV) of loading buffer.
   c. Wash column with 5 CV of elution buffer.
   d. Equilibrate with 5-10 CV of loading buffer.
   e. Load sample.
   f. Wash with at least 5 CV of loading buffer or until the OD_{280} reading returns to baseline level.
   g. Elute with chosen elution buffer, stepwise or gradient. (See Optimization, Elution page 7)
   h. Strip with 5-10 CV of elution buffer containing 1.0-1.5 M NaCl.
   i. Re-equilibrate with 5 CV loading buffer.

2. Optimization
   a. All buffers that contact the protein sample must be known to maintain protein solubility for all proteins in the sample at the time of exposure to those buffers. If there are any doubts, this should be tested in advance to avoid protein loss and fouling of the column.
   b. Method optimization analysis
      – Analyze flow through samples to ensure that target protein(s) is captured under the chosen conditions and binding capacity for target protein(s), with the test protocol, is not exceeded.
      – Analyze all effluents to determine highest purity and activity of target molecule (for example by ELISA, UV_{280}, SDS-Page, or other appropriate techniques).

Instructions for Use – Automated or Pumped Chromatography Systems (continued)

c. Protein loading conditions and buffer pH
   – Choose or test buffers with sufficient buffer range and capacity for the chosen resin and sample (all proteins remain soluble).
   – If the pI of the target molecule is known, start with a much narrower pH range, about 1 pH unit ± the known pI.
   – Collect flow through sample for analysis. These samples can be used to confirm efficient capture of the target protein(s) under the test conditions.

d. Elution
   – An ion exchange elution buffer is generally the same as the loading buffer with additional salt. However, pH elution can be used (decrease pH for anion exchange, increase pH for cation exchange) as long as protein solubility is not an issue.
   – Linear gradient
      • If the exact elution point of the target molecule is unknown or more than one protein is to be purified, a linear gradient can be used to elute the target protein(s).
      • A longer gradient (more volume used for concentration change) with a shallow slope will result in better separation of proteins in a larger volume (broad peaks). A shorter, steeper slope will result in sharper peaks with less resolution.
   – Step elution
      • Stepwise elution can be used to simplify a purification process. However, it is important to know the elution behavior of the target and impurity proteins before designing this procedure. This type of elution is typically takes less time then a gradient elution.
      • A common step protocol has three post-protein capture steps.
         a) After protein capture (loading step), the column is washed with elution buffer containing some salt, but not enough to elute the target protein. Wash to baseline OD_{280}, typically 5 CV.
         b) Then the salt concentration is increased just enough to elute the target protein and as few other proteins as possible. Elute to baseline.

c) Finally the highest salt concentration is used to strip the remaining proteins from the column. Wash to baseline.

d) If more then one protein is to be purified, a second elution step can be added before the high salt column strip.

– Collect all effluents (initially elute using a linear gradient to 100% of your elution buffer usually over 10 CV, unless data suggests otherwise) for analysis

3. Determination of binding capacity (DBC)
   a. Procedure
      – System Parameters:
      - Flow rate: 200 cm/hr (or intended flow rate)
      - Equilibration: 5 CV loading buffer
      - Sample load: 30 mL injection of 5 mg/mL of protein (using sample pump)
      - Wash: 20 CV loading buffer
      - Clean: 5 CV elution buffer
      - Re-equilibration: 5 CV loading buffer
      - Void volume (V_0): To determine V_0, run the procedure using conditions which prevent protein binding. For example, use an elution buffer instead of a loading buffer for the equilibration and sample loading steps.

      – Calculation
      - DBC Formula:
        $DBC = C \times (V_L - V_0)$
        C = Concentration of load
        V_L = Volume at 10% or 50% breakthrough
        V_0 = From the void volume determination described above, V_0 is the total volume passing through the system from the time of injection (0% deflection of OD_{280}) until protein breakthrough (increase in OD_{280}).

   b. **Note:** Same protocol can be used for binding capacity determination for other proteins and/or different buffers.

Instructions for Use – Automated or Pumped Chromatography Systems (continued)

4. Cleaning
   a. Column performance may decline over time due to incomplete removal of proteins or contaminates. They can be easily removed by using the following procedure:
      1. Wash column with 10 CV of loading buffer
      2. Wash column with 5 CV of elution buffer
      3. Wash column with 10 CV of loading buffer
      4. Wash column with 5 CV of 1 M NaOH
      5. Wash column with 10 CV of loading buffer or until the conductivity has returned to the level of the loading buffer
5. Storage
   a. The column must be stored at 2-8 °C and cannot be frozen.
      Between runs, store the column at 2-8 °C in loading buffer.
      The storage buffer may also contain bacteriostatic agents such as 20% (v/v) ethanol and/or 1 M NaCl.

Instructions for Use – Manual Use with Syringe

Materials Required

- Syringes (5-30 mL) with luer lock fittings (larger syringes have less pressure)
- Filtered buffers (see tables, page 5, 6)

Syringe Protocol

Note: It is important to avoid introducing air into the column. Remove air bubbles from fluid filled syringe before attachment to the column each time the syringe is changed.

Note: When pushing fluid through the syringe, maintain a relatively constant flow rate with minimal backpressure, typically 1-4 mL/min.

1. Fill the syringe with loading buffer. To avoid getting air into the column, load syringe with more than the required amount of buffer.
2. Equilibrate the column with 5-10 column volume (CV) of loading buffer.
   – Secure the filled syringe to the column luer connector. Check that there are no air bubbles at the site of attachment.
   – Apply gentle pressure to push the buffer through the device.

Instructions for Use – Manual Use with Syringe (continued)

3. Fill syringe with sample.
   – Adjust sample pH or concentration as necessary for the separation.
4. Load sample onto the column avoiding the introduction of air bubbles.
   – Collect all effluent in order to capture any breakthrough or sample analysis.
5. Wash column with 5 CV of loading buffer to wash off any unbound sample.
   – Collect all effluent in order to capture any breakthrough.
6. Fill syringe with elution buffer. Secure the filled syringe to the column luer connector. Check that there are no air bubbles at the site of attachment.
7. Run 10-20 CV of elution buffer through the column to elute bound proteins.
   – If eluting more then one target protein, and ionic strength for each elution is known, a step elution method is recommended.
   – Collect all effluent containing eluted protein(s).
8. If the column will be reused, strip residual protein with 5 CV of 1 M NaCl in loading buffer.
9. Fill the syringe with loading buffer. Equilibrate the column with 5-10 CV of loading buffer.

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