Marvelgent biosciences

Store product at 2°C – 8°C. Do not freeze. The product is shipped at ambient temperature.

ProteIndex™ Ion Exchange Media

Q Agarose 6 Fast Flow

SP Agarose 6 Fast Flow

DEAE Agarose 6 Fast Flow

CM Agarose 6 Fast Flow

Intended Use

This product is intended for research use only. Not intended for any animal or human therapeutic or diagnostic use.

Cat. No.	Package Size
11-0232-025	ProteIndex IEX-Q Agarose 6 Fast Flow, 25 mL settled resin
11-0232-300	ProteIndex IEX-Q Agarose 6 Fast Flow, 300 mL settled resin
11-0234-025	ProteIndex IEX-SP Agarose 6 Fast Flow, 25 mL settled resin
11-0234-300	ProteIndex IEX-SP Agarose 6 Fast Flow, 300 mL settled resin
11-0236-025	ProteIndex IEX-DEAE Agarose 6 Fast Flow, 25 mL settled resin
11-0236-300	ProteIndex IEX-DEAE Agarose 6 Fast Flow, 300 mL settled resin
11-0238-025	ProteIndex IEX-CM Agarose 6 Fast Flow, 25 mL settled resin
11-0238-300	ProteIndex IEX-CM Agarose 6 Fast Flow, 300 mL settled resin

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1. Product Description

PROTEINDEX™ Ion Exchange Media CM, SP, DEAE, Q Agarose 6FF are specifically developed for high-resolution separation of proteins, peptides, nucleic acids and other charged biomolecules according to their difference in surface charge. The base matrix of PROTEINDEX™ IEX-CM, IEX-SP, IEX-DEAE, IEX-Q Agarose 6 Fast Flow is highly 6% cross-linked agarose to which charged groups are attached, which gives the ion exchangers high chemical and physical stability. The type of charged group determines the type and strength of the exchanger, while the total number and availability of the charged groups determine the capacity.

Sulfonic and quaternary amines form strong ion exchangers, which are completely ionized over a broad pH range. All others form weak ion exchangers, where the degree of dissociation, and thus the exchange capacity, varies markedly with pH. "Strong" and "weak" refer to the extent of ionization with pH, and not to the strength of binding.

Marvelgent ion exchanger media are optimized for high sample loading capacity with reliable, reproducible performance, suited for process scale chromatography. The characteristics such as capacity, elution behavior and pressure/flow rate, as shown in table 1 and table 2, are unaffected by the solutions commonly used in process chromatography and cleaning procedures.

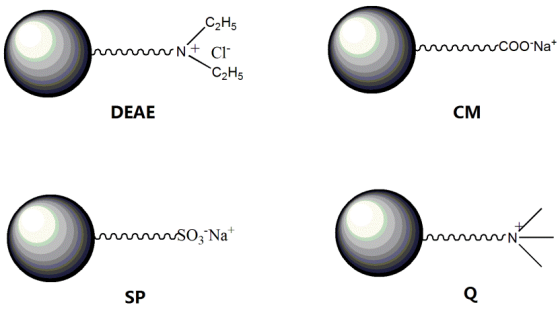


Figure 1. Structures of ion exchange media IEX-CM, IEX-SP, IEX-DEAE, IEX-Q Agarose 6 Fast Flow.

2. Characteristics of Ion Exchangers Agarose 6 Fast Flow

IEX-CM Agarose 6 Fast Flow is a weak cation exchanger. The ion exchange group is a carboxy methyl group:

$$-O-CH_2COO-$$

IEX-SP Agarose 6 Fast Flow is a strong cation exchanger. The ion exchange group is a sulphopropyl group:

$$-O-CH_2CHOHCH_2OCH_2CH_2CH_2SO_3^-$$

IEX-DEAE Agarose 6 Fast Flow is a weak anion exchanger. The ion exchange group is a diethylaminoethyl group:

$$-O-CH_2CH_2-N^+(C_2H_5)_2H$$

IEX-Q Agarose 6 Fast Flow is a strong anion exchanger. The ion exchange group is a quaternary amine group:

$$-O-CH_2CHOHCH_2OCH_2CHOHCH_2N^+(CH_3)_3$$

Table 1. Characteristics of IEX-CM and SP Agarose 6 Fast Flow.

Cation Exchanger	CM Agarose 6 FF	SP Agarose 6 FF
Matrix	Highly cross-linked 6% agarose	
Ion exchange type	Weak cation	Strong cation
Total ionic capacity	0.09 - 0.13 mmol H^+ /mL medium	0.18 - 0.25 mmol H^+ /mL medium
Particle Size (µm)	45 – 165	
Flow rate	300-600 cm/h	400-700 cm/h
pH stability	4 – 13	
Storage buffer	20% ethanol	20% ethanol，0.2 M sodium acetate
Storage	2°C - 30°C	

Table 2. Characteristics of IEX-DEAE and Q Agarose 6 Fast Flow.

Anion Exchanger	DEAE Agarose 6 FF	Q Agarose 6 FF
Matrix	Highly cross-linked 6% agarose	
Ion exchange type	Weak anion	Strong anion
Total ionic capacity	0.11 – 0.16 mmol Cl^- /mL medium	0.18 – 0.25 mmol Cl^- /mL medium
Particle Size (µm)	45 – 165	
Flow rate	300-600 cm/h	400-700 cm/h
pH stability	2 – 12	
Storage buffer	20% ethanol	
Storage	2°C - 30°C	

3. Operation

3.1 Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter buffers by passing them through a 0.22 µm or 0.45 µm filter before use.

3.2 Sample Preparation

It is recommended to filter the sample solutions by passing them through a 0.22 µm or 0.45 µm filter before use.

3.3 Packing Columns

1. Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.
2. Close the column outlet leaving the net covered with packing buffer.
3. Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass

rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

4. Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, the medium is packed at a constant pressure of approximately 1 bar (0.1 MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25°C, low viscosity buffer).If the recommended pressure or flow velocity cannot be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.
5. When the bed has stabilized, close the bottom outlet and stop the pump.

   If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.
6. With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.
7. Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.
8. Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

3.4 Column Packing Evaluation

When column packing is complete, equilibrate the column with up to 5 CV equilibration buffer. To test the effectiveness of column packing, inject a sample of a low molecular weight, unrestrained compound (for example, acetone or 1M NaCl). If acetone is used as the test marker (use a conductivity monitor), then the equilibration buffer salt concentration should be 100 – 200 mM.

The sample volume should be 1-3% of the total column volume. Column testing should be operated using the same linear velocity used to load and /or elute the sample.

To obtain comparable height equivalent to a theoretical plate (HETP) values among columns, the same conditions must be applied. Minimum theoretical plate values should be 1,000 - 3,000 plates/m for linear velocities of 50 - 500 cm/hr.

HETP=L/N

$N=5.54(V_e/W_{1/2h})^2$

L=Bed height (cm)

N= Number of theoretical plates

V_e=Peak elution volume or time

$W_{1/2h}$=Peak width at peak half height in volume or time

V_e and $W_{1/2h}$ should always be in the same units

Peaks should be symmetrical and the asymmetry factor as close as possible to 1. Values of 0.8-1.8 are acceptable.

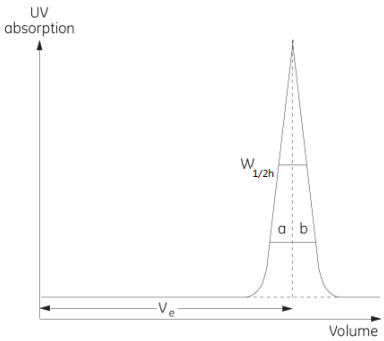
Peak asymmetry factor calculation:

$A_s=b/a$

a= Front section of peak width at 10% of peak height bisected by line denoting V_e

b=Latter section of peak width at 10% of peak height bisected by line denoting V_e

A_s=0.8-1.8 is acceptable.



3.5 Sample Purification

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column. Remove the snap-off end at the column outlet.
2. Wash the column with 10 column volumes of binding buffer.
3. Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.
4. Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.
5. Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

3.6 Analysis

Identify fractions from individual steps using UV absorbance, SDS-PAGE, or western blot.

4. Regeneration

After each separation, elute any reversibly bound material either with a high ionic strength solution (e.g. 1M NaCl in buffer) or by increasing pH. Regenerate the medium by washing it with at least 5 bed volumes of the regeneration buffer, or until the column effluent obtains stable conductivity and pH values.

5. Cleaning-in-Place

Cleaning-in-Place (CIP) is a cleaning procedure that removes contaminants such as lipids, precipitates, or denatured proteins that may remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the media bed and helps to maintain the capacity, flow properties and general performance of the media.

A specific CIP protocol should be designed for each process according to the type of contaminants present. CIP cycle is generally recommended every 1-5 separation cycles.

To remove ionically bound proteins:

Wash with 1 column volumes of 2 M NaCl. Contact time 10 – 15 min.

To remove precipitates, hydrophobically bound proteins and lipoproteins:

Wash with at least 2 column volumes of 1 M NaOH. Contact time 1 – 2 h.

To remove lipids and very hydrophobic proteins:

Wash with 3 column volumes of 0.5% non-ionic detergent (e.g.1 M acetic acid), 70% ethanol or 30% isopropanol. Contact time 1 – 2h.

6. Storage

Store the IEX-SP Agarose 6 Fast Flow in 20% ethanol contains 0.2M sodium acetate at 2-30°C. Store the IEX-CM Agarose 6 Fast Flow, IEX-DEAE Agarose 6 Fast Flow and IEX-Q Agarose 6 Fast Flow in 20% ethanol at 2-30°C.

7. Trouble Shooting Guide

Problem	Probable Cause	Solution
Back pressure is too high	Column is clogged	Cleaning in place (Section 5).
	Sample solution contains precipitate	Filtering the sample solution by passing them through a 0.22 μm or 0.45 μm filter.
Eluate is not pure	The medium repeat too much times.	Cleaning in place (Section 5).
	Wash is not enough.	Increase the volume of Wash Buffer.

8. Related Products

Product Name	Package Size	Cat. No.
ProteIndex IEX-Q Agarose 6 FF Prepacked Cartridge		
	5x1 mL	11-0233-5x1
	1x5 mL	11-0233-1x5
	5x5 mL	11-0233-5x5
ProteIndex IEX-SP Agarose 6 FF Prepacked Cartridge		
	5x1 mL	11-0235-5x1
	1x5 mL	11-0235-1x5
	5x5 mL	11-0235-5x5
ProteIndex IEX-DEAE Agarose 6 FF Prepacked Cartridge		
	5x1 mL	11-0237-5x1
	1x5 mL	11-0237-1x5
	5x5 mL	11-0237-5x5
ProteIndex IEX-CM Agarose 6 FF Prepacked Cartridge		
	5x1 mL	11-0239-5x1
	1x5 mL	11-0239-1x5
	5x5 mL	11-0239-5x5

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