Fractogel® AEX screening kit

Cat. No. 1.50502.0001

1. Introduction

Fractogel® EMD resins are synthetic methacrylate based polymeric beads with pores of about 800 Å and excellent pressure stability resulting in high flow rate capabilities. The surface is hydrophilic due to ether linkages in the polymer matrix. The media are available in two particle sizes: M (40-90 µm) and S (20-40µm)- for both production (M) and high-resolution polishing applications (S). Ion exchange media are available with different ligands for weak or strong cation and anion exchange chromatography.

Available functional groups:		
strong basic		
TMAE	(trimethylammoniumethyl)	pK > 13
weak basic		
DEAE	(diethylaminoethyl)	pK 9,5 - 11
DMAE	(dimethylaminoethyl)	pK 8 - 9
strong acidic		
SO_3^-	(sulphoisobutyl)	pK < 1
SE	(sulphoethyl)	pK < 1
weak acidic		
COO^-	(carboxy)	pK 4,5

Fractogel® EMD process media provide high capacity and resolution along with all the advantages of polymer chromatographic beads such as high throughput, easy column packing, long life time and high mechanical stability.

Long, linear polymer chains ("tentacles") carry the functional groups. All tentacles are covalently attached to the methylacrylate backbone and are chemically stable under conditions applied during chromatography, regeneration and sanitization. The most important advantage of the tentacle chemistry is the large number of sterically accessible ligands for the binding of biomolecules.

Due to the tighter binding of the target molecule, the capture or initial step in a purification is often more efficient than with other resins. This results in higher recovery of valuable biomolecules. The cleanability of Fractogel® EMD ion exchangers assures long column lifetime.

The Fractogel® AEX screening kit is a tool that offers a fast and convenient way to determine which anion exchange ligand is best suited for a given application. The kit consists of four scout columns, prepacked with different anion exchange materials.

1.50502.0001	Fractogel® AEX screening kit
including:	
Fractogel® DMAE scout column	1 x 1 ml
Fractogel® DEAE scout column	1 x 1 ml
Fractogel® TMAE scout column	1 x 1 ml
Fractogel® TMAE Hicap scout column	1 x 1 ml
Connectors:	
LuerLock female/ 1/16" male	4 pieces

Fractogel® scout columns are designed for the rapid evaluation of Fractogel® resins for bioprocess development. The results obtained for biomolecule separation using Fractogel® scout columns are generally comparable to the results achieved using laboratory-scale columns (e.g. 100 x 16 mm, for details see chapter 6, Functional testing of Fractogel® scout columns and chapter 7, Screening for experimental conditions using Fractogel® scout columns.

Thus, in bioprocess design, the scout columns can be used to rapidly determine whether a target biomolecule is bound to and can be eluted from a given Fractogel® EMD resin. Additionally, data from experiments using Fractogel® scout columns allow one to determine whether an appropriate target yield may be achieved and whether major contaminants could potentially be removed. After choosing the optimal Fractogel® EMD resin, bulk media are available for larger scale preparative work.

2. Guidelines for use of Fractogel® scout columns

The Fractogel® scout columns are delivered in a buffer comprised of 0.005 % (w/v) Triton X 100, 150 mM NaCl and stabilized with ProClin as a preservative. Prior to the first usage the preservation buffer must be completely removed by washing the column with at least 5 column volumes (5 ml) of starting buffer.

For storage of Fractogel® scout columns after usage rinse the columns with three column volumes of 20 % ethanol in 150 mM NaCl (3 ml).

All buffers should be filtered using a 0.22 µm filtration unit to remove particles. All protein solutions should

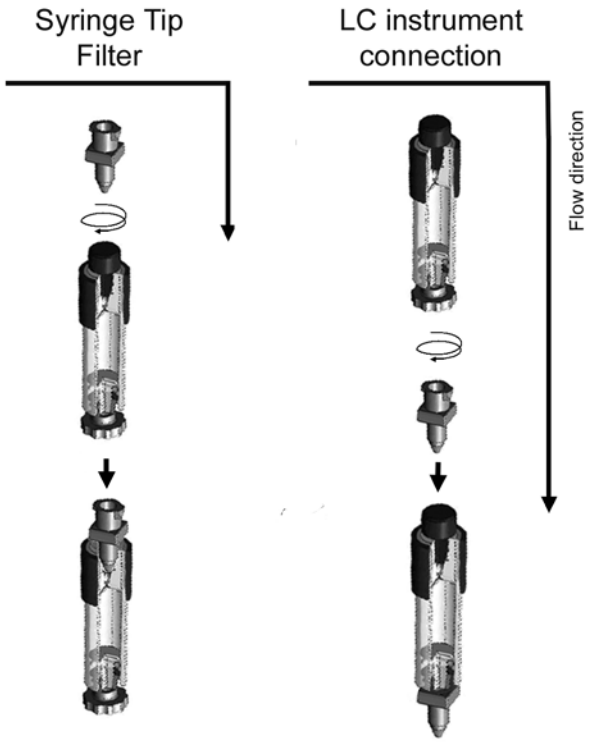
be filtered using a 0.45 µm filtration unit prior to injection
Do not operate the Fractogel® scout column at linear flow rates above 600 cm/hour (5.2 ml/min).

The characteristics of the Fractogel® scout columns are summarized in Table 1. For experimental setup, please additionally refer to section 5, Gel properties and to section 8, Biological Buffers.

Table 1: Characteristics of Fractogel® scout columns

Column volume	1 ml
Column dimension	0.82 x 1.92 cm
Maximum Flow Rate	600 cm/hour (5,2 ml/min)
Operating Flow Rate	80 - 250 cm/hour
Maximum back pressure	0.5 Mpa, 5 bar
Column Pressure Drop	~ 0.1 bar at 180 cm/hour ~ 0.5 bar at 500 cm/hour
Chemical stability	All commonly used aqueous buffers,
(do not store columns under these conditions)	1 M NaOH 7 M urea 6 M guanidine hydrochloride 70 % ethanol

* Please acknowledge that the chemical stability of the respective resin can be different



The scout columns provided with the Fractogel® AEX screening kit are manufactured from polypropylene, which is biocompatible and non-interactive with biomolecules. The scout columns are delivered with a stopper at the inlet and a Luerlock stopper at the outlet. The column hardware is designed to allow for both manual and LC-instrument controlled usage. When used manually, the scout columns are operated as syringe-tip filters, when used with LC-instrument control, the scout columns can be connected using the provided 1/16" adapters.

The Fractogel® scout columns cannot be opended or refilled.

To prevent leakage it is essential to ensure that the adaptors are tightly connected to the column.

3. Storage Conditions

For long term storage the kit should be stored at 4° - 8°C.

Avoid freezing the scout columns!

For storage of Fractogel® scout columns after usage rinse the columns with three column volumes of 20 % ethanol in 150 mM NaCl (3 ml).

4. Practical hints

Selection of ion exchanger
Ion exchange chromatography is based on the amphoteric behaviour of proteins. At low acidic pH-values, all amino acids are cations due to protonation of the NH_2-groups and undissociated COOH-groups resulting in a net positive charge. At alkaline pH-values above pH 12, the amino-groups are uncharged, whereas the carboxyl-groups will be ionized, resulting in a negatively charged molecule.

Biomolecules are bound to ion exchangers when they carry a net charge opposite to the surface charge of the ion exchanger. The pH value at which a biomolecule has no net charge is called the isoelectric point (pI). At buffer pH values below the pI, a given biomolecule will carry a positive net charge and will bind to cation exchangers (Fractogel® EMD SO_3^-, COO^- and SE Hicap). At buffer pH values above the pI, the respective biomolecule will carry a net negative charge and will bind to anion

exchangers (Fractogel® EMD TMAE, TMAE Hicap, DEAE and DMAE). The charge/pI relationship for the target biomolecule across the pH range of interest can be determined from the titration curve (compare Fig. 1).

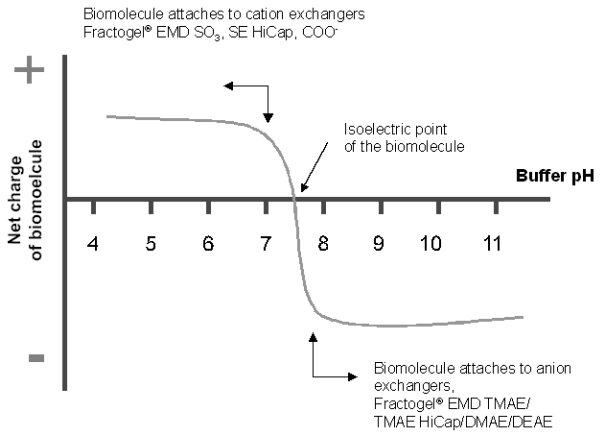


Fig. 1. The net charge of a biomolecule as a function of the buffer pH value

If the target biomolecule is most stable below the pI value, a cation exchanger should be used. If stability of the target biomolecule is higher at buffer pH values above its pI, an anion exchanger should be used. When the biomolecule is stable over a wide range of buffer pH values, either type of ion exchange can be used for purifications either alone or in subsequent combinations. It is important in ion exchange chromatography to choose optimal pH conditions so that the ion exchange groups are ionized. On the other hand, the buffer pH-value must be sufficiently different from the isoelectric points of the target biomolecule in order to maintain sufficient net charge on the surface. Applying an increasing salt gradient or changing the pH of the buffers will reduce binding to the gel and elute proteins successively from the column when the charge differences have been neutralized.

NOTE:
For pK values of selected biological buffers and buffering ranges please refer to the appendix

Starting condition for ion exchange chromatography:
Highest salt concentration that permits binding of the target protein.

Please equilibrate the Fractogel® scout columns with at least 5 column volumes of starting buffer prior to application of the sample.

NOTE:
It is strongly recommended that a typical run gradient without sample application is perfomed prior to the first use of a Fractogel® scout column to ensure removal of preservative compounds and to achieve a stable base line.

Washing step:
An ionic strength higher than the loading step and less than the elution step will remove contaminants that bind to the column.

Elution condition:
Lowest ionic strength which allows the elution of the protein of interest. In case of weak anion or cation exchange chromatography, changing the buffer pH can also be used to elute the target biomolecule.

Stripping Step
A higher concentration of salts in the buffer than that used for elution is recommended for stripping the column after use.

Cleaning and regeneration:

Each column should be cleaned thoroughly after the separation step or at least after the experiments have been finished (especially if the gel bed has changed colour or the back pressure increased during the separation).

Various methods are available for cleaning chromatographic media. Synthetic polymeric matrices are characterized by higher chemical stability than inorganic sorbents based on silica gels, which are unstable in the presence of NaOH. In contrast to media based on carbohydrates, synthetic polymeric matrices can also withstand treatment with acids.

Lipids or lipoproteins can be removed with organic solvents like ethanol, isopropanol or ethylene glycol. Denatured proteins can be effectively removed with sodium hydroxide (0.1 N up to 1 N NaOH). In addition to bases and acids, organic solvents can be used.

If contaminants are tightly bound, it may be necessary to clean the column with an acidic pepsin solution (0.1% pepsin in 0.01 N HCl), 6 M guanidine hydrochloride or diluted sodium lauroyl sarcosinsate solution (2% SLS in 0.25 M NaCl). The removal of SLS is achieved by treatment with 20% 2-propanol in 0.01N HCl.

After cleaning and regeneration, Fractogel® scout columns must either be rinsed with three column volumes of 20 % ethanol in 150 mM NaCl (3 ml) for storage or reequilibrated for the next chromatographic run.

Avoid storage of columns in cleaning solutions.

Table 2. Recommended cleaning and regeneration steps

Reagent	Concentration	Bed volumes
NaCl	up to 2 M	2 BV
NaOH	0.1 - 1 N	1 - 5 BV
HCl	1 - 2 N	1 - 5 BV
Pepsin/HCl	0.1% / 0.01N HCl	1 - 2 BV
SLS/NaCl	2% / 0.25 M NaCl	1 - 2 BV
Isopropanol	20 %	1 - 2 BV
Acetonitrile	20 %	1 - 2 BV
Others	20 % ethanol guanidine HCl, 6 M urea	1 - 2 BV

5. Gel properties

Please find below a table summarizing the properties of the Fractogel® EMD anion exchange materials provided with the Fractogel® AEX screening kit.

The provided scout columns are packed with the respective (M)-type gel at a compression rate of 17 %.

Fractogel® EMD DMAE

Cat. No.:	1.16884 (M-type))
	1.16889 (S-type)
Bulk material	100 ml, 500 ml, 5 l
Particle size	40 – 90 µm (M)
	20 – 40 µm (S)
Type of chromatography	Weak anion exchange chromatography
Pressure drop	< 1 bar (5 ml/min)
Pressure limit	8 bar
Functional group	Dimethylaminoethyl group (DMAE)
Protein binding capacity	~ 100 mg BSA/ml of gel
pK value	~ 10 §
pH stability range	pH 2 up to pH 13
Elution conditions	High salt concentrations; Changing of buffer pH
Operating temperature	4 °C to room temperature
Regeneration	1 – 2 M NaCl
Sanitization	0.1 – 0.5 M NaOH, sodium lauroyl sarcosinate
Linear flow rate	Up to 360 cm/hour (S-type)
	Up to 600 cm/hour (M-type)$

§ Due to the titration behaviour the ion exchange capacity of Fractogel® EMD DMAE can be used from pH 2 up to pH 8.5.

Fractogel® EMD DEAE

Cat. No.:	1.16883 (M-type)
	1.16888 (S-type)
Bulk material	100 ml, 500 ml, 5 l
Particle size	40 – 90 µm (M)
	20 – 40 µm (S)
Type of chromatography	Weak anion exchange chromatography
Pressure drop	< 1 bar (5 ml/min)
Pressure limit	8 bar
Functional group	Diethylaminoethyl-group
Protein binding capacity	~ 130 mg lysozyme /ml of gel
pK value	> 11 §
pH stability range	pH 2 up to pH 13
Elution conditions	High salt concentrations; Changing of buffer pH
Operating temperature	4 °C to room temperature
Regeneration	1 – 2 M NaCl
Sanitization	0.1 – 0.5 M NaOH, sodium lauroyl sarcosinate
Linear flow rate	Up to 360 cm/hour (S-type)
	Up to 600 cm/hour (M-type)$

§ Due to the titration behaviour the ion exchange capacity of Fractogel® EMD DEAE can be used from pH 2 up to pH 9.5.

Fractogel® EMD TMAE

Cat. No.:	1.16881 (M-type)
	1.16887 (S-type)
Bulk material	100 ml, 500 ml, 5 l
Particle size	40 – 90 µm (M)
	20 – 40 µm (S)
Type of chromatography	Strong anion exchange chromatography
Pressure drop	< 1 bar (5 ml/min)
Pressure limit	8 bar
Functional group	Trimethylammoniumethyl –group (TMAE); “Q-type”
Protein binding capacity	~ 100 mg BSA/ml of gel
pK value	> 13
pH stability range	pH 2 up to pH 12
Elution conditions	High salt concentrations
Operating temperature	4 °C to room temperature
Regeneration	1 – 2 M NaCl
Sanitization	0.1 – 0.5 M NaOH, sodium lauroyl sarcosinate
Linear flow rate	Up to 360 cm/hour (S-type)
	Up to 600 cm/hour (M-type)$

§ Due to the titration behaviour the ion exchange capacity of Fractogel® EMD TMAE can be used from pH 2 up to pH 12.

Fractogel® AEX screening kit

Cat. No. 1.50502.0001

Fractogel® EMD TMAE Hicap

Cat. No.:	1.10316
Bulk material	100 ml, 500 ml, 5 l
Particle size	40 – 90 µm (M)
Type of chromatography	Strong anion exchange chromatography
Pressure drop	< 1 bar (5 ml/min)
Pressure limit	8 bar
Functional group	Trimethylammoniumethyl –group (TMAE); "Q-type"
Protein binding capacity	~ 180 mg lysozyme /ml of gel
pK value	> 13
pH stability range	pH 2 up to pH 12
Elution conditions	High salt concentrations,
Operating temperature	4 °C to room temperature
Regeneration	1 – 2 M NaCl
Sanitization	0.1 – 0.5 M NaOH, sodium lauroyl sarcosinate
Linear flow rate	Up to 600 cm/hour[$]

[§] Due to the titration behaviour the ion exchange capacity of Fractogel® EMD TAME Hicap can be used from pH 2 up to pH 12.

[$] Recommended flow rates in large production scale columns can be lower (up to 250 cm in columns with > 50 cm inner diameter)

6. Functional testing of Fractogel® scout columns

The Fractogel® scout columns have been thoroughly tested with regard to chromatographic performance and reproducibility using model protein mixtures. The results have also been compared to data on model protein separations obtained with laboratory-scale columns (100 x 16 mm) under scaled loading parameters and equal linear flow rate to allow for the comparison of resolution factors and elution conductivity.

NOTE:
Please understand the presented data as a hint to judge results obtained by initial screening experiments with Fractogel® scout columns prior to upscaling for more labour-intensive experiments using laboratory-scale columns.

The Fractogel® scout columns were tested for performance by separating model protein mixtures using the following gradient method. All tests have been performed at room temperature using a linear flow rate of 78 cm/hour or 0.67 ml/min, respectively.

Sample: Conalbumin 4 mg/ml;
Human serum albumin (HSA) 5 mg/ml

The proteins were dissolved in 20 mM Tris pH 8,0

Injection:	25 µl sample volume	
Detection:	Absorbance at 280 nm	
Eluents:	Buffer A: 20 mM Tris pH 8,0	
	Buffer B: 20 mM Tris pH 8,0, 1 M NaCl	
Gradient:	0 min/0 %	0,26 ml/min
	1 min/1 % B	0,26 ml/min
	7 min/7 % B	0,67 ml/min
	50 min/50 % B	0,67 ml/min
	50,1 min/100 % A	0,67 ml/min
	65,0 min/100 % A	0,67 ml/min (10 CV)

Resolution factors (R) for the separation of neighbouring peaks were calculated for all experiments according to EUP standard following equation I:

$$(I) \qquad R = \frac{1.18(t_2 - t_1)}{W_{1(1/2)} + W_{2(1/2)}}$$

where $W_{1(1/2)}$ and $W_{2(1/2)}$ are the peak widths at half the maximum height and t_1 and t_2 are the retention times of the respective peaks.

Fractogel® EMD DMAE (M)

Using Fractogel® DMAE scout columns, the two model proteins were separated as documented in figure 2 A. The representative UV trace for three independent experiments is shown.

As a comparison, the same proteins were separated under identical conditions using a laboratory-scale column packed with Fractogel® EMD DMAE (M) (dimension 100 x 16 mm), however the sample load was increased 20-fold to maintain the scale. A representative UV-trace of this separation is shown in Fig. 2 B with the same peak assignment.

Resolution factors were calculated for the separation of conalbumin and HSA as follows: 1.94 for scout columns and 1.96 for laboratory-scale columns. The data indicates that, using the 1 ml- Fractogel DMAE scout columns for model protein mixtures, equal resolution as with a 100 x 16 mm-laboratory-scale column is achievable.

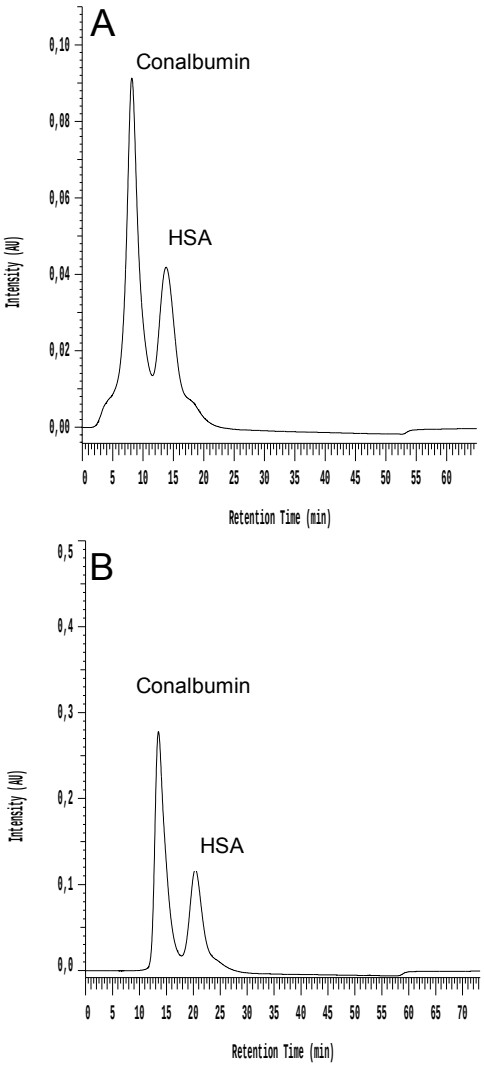


Fig 2. Representative UV-traces of the separation of a mixture of conalbumin and HSA on Fractogel® DMAE scout columns (**A**) or laboratory scale columns (**B**, dimensions: 100 x 16 mm). For details see text.

Elution conductivities in mS/cm on Fractogel® EMD DMAE (M) for the respective model proteins are summarized in table 3. The elution conductivities for the two tested model proteins on Fractogel® DMAE scout columns were reproducibly found to be approximately 23 % lower as compared to the laboratory-scale columns (dimension 100 x 16 mm) in case of conalbumin and 20 % lower in case of HSA, respectively. Thus elution conductivity of a given target biomolecule on a laboratory-scale column (100 x 16 mm) can be predicted to be ~ 20 % higher as in case of the scout column provided the described experimental setup is maintained.

Table 3. Elution conductivities of model proteins on Fractogel® EMD DMAE (M).

Protein	Fractogel® DMAE scout column	Fractogel® EMD DMAE (M) column (100 x 16 mm)
Conalbumin	4.3 ± 0.05	5.6 ± 0.02
Human Serum Albumin	10.5 ± 0.06	13.1 ± 0.1

Fractogel® EMD DEAE (M)

Using Fractogel® DEAE scout columns, conalbumin and HSA were separated as documented in figure 3 A.
The representative UV trace for three independent experiments is shown. As a comparison, the same proteins were separated under identical conditions using a laboratory-scale column packed with Fractogel® EMD DEAE (dimension 100 x 16 mm), however sample load was increased 20-fold to maintain the scale. A representative UV-trace of this separation is shown in Fig 3 B with the same peak assignment.

Resolution factors for the separation of conalbumin and HSA were as follows: 1.9 for scout columns and 2.5 for laboratory-scale columns. The data indicates that, using the 1 ml- Fractogel® DEAE scout columns for model protein mixtures, calculated resolution remains approximately 75 % of the resolution of a 100 x 16 mm-laboratory-scale column.

Table 4. Elution conductivities of model proteins on Fractogel® EMD DEAE (M)

Protein	Fractogel® DEAE scout column	Fractogel® EMD DEAE (M) column (100 x 16 mm)
Conalbumin	7.9 ± 0.05	10.1 ± 0.07
Human Serum Albumin	19 ± 0.01	22 ± 0.04

Elution conductivities in mS/cm on Fractogel® EMD DEAE (M) for the respective model proteins are summarized in table 4. The elution conductivities for the two tested model proteins on Fractogel® DMAE scout columns were reproducibly found to be approximately 22 % lower as compared to the laboratory-scale columns (dimension 100 x 16 mm) in case of conalbumin and 16 % lower in case of HSA, respectively. Thus elution conductivity of a given target biomolecule on a laboratory-scale column (100 x 16 mm) can be predicted to be ~ 20 % higher

as in case of the scout column provided the described experimental setup is maintained.

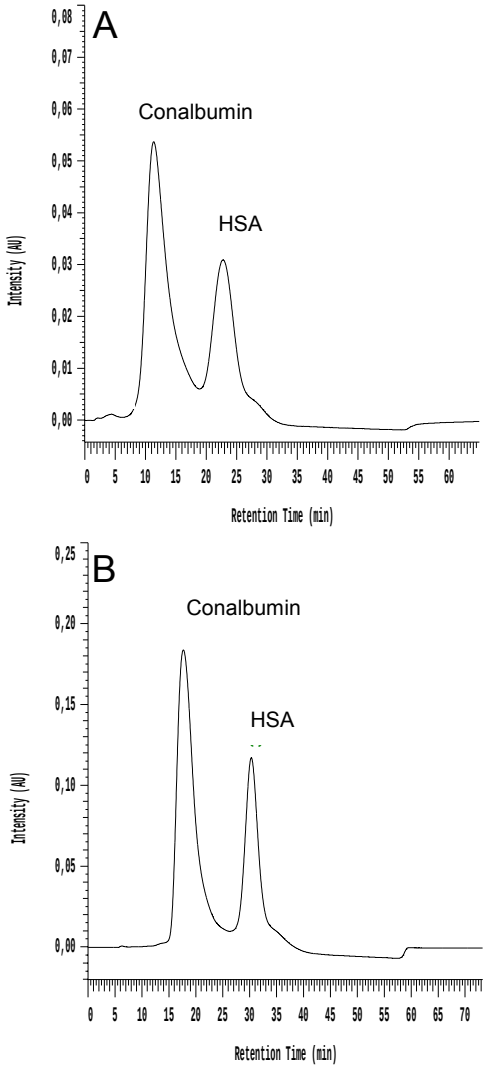


Fig 3. Representative UV-traces of the separation of a mixture of conalbumin and HSA on Fractogel® DEAE scout columns (**A**) or laboratory scale columns (**B**, dimensions: 100 x 16 mm). For details see text.

Fractogel® EMD TMAE (M)

Using Fractogel® TMAE scout columns, conalbumin and HSA were separated as documented in figure 4A. The representative UV trace for three independent experiments is shown. As a comparison, the same proteins were separated under identical conditions using a laboratory-scale column packed with Fractogel® EMD TMAE (M) (dimension 100 x 16 mm), however sample load was increased 20-fold to maintain the scale. A representative UV-trace of this separation is shown in Fig 4 B.

Resolution factors for the separation of conalbmuin and HSA were as follows: 1.9 for scout columns and 2.0 for laboratory-scale columns. The data indicates that, using the 1 ml- Fractogel® TMAE scout columns for model protein mixtures, calculated resolution remains appromimately equal as compared to the resolution of a 100 x 16 mm-laboratory-scale column.

Table 5. Elution conductivities of model proteins on Fractogel® EMD TMAE (M)

Protein	Fractogel® TMAE scout column	Fractogel® EMD TMAE (M) column (100 x 16 mm)
Conalbumin	10.7 ± 0.025	13 ± 0.15
Human Serum Albumin	20.4 ± 0.2	23.4 ± 0.1

Elution conductivities in mS/cm on Fractogel® EMD TMAE (M) for the respective model proteins are summarized in table 5. The elution conductivities for the two tested model proteins on Fractogel® TMAE scout columns were reproducibly found to be approximately 18 % lower as compared to the laboratory-scale columns (dimension 100 x 16 mm) in case of conalbumin and 13 % lower in case of HSA, respectively. Thus elution conductivity of a given target biomolecule on a laboratory-scale column (100 x 16 mm) can be predicted to be ~ 15 % higher as in case of the scout column provided the described experimental setup is maintained.

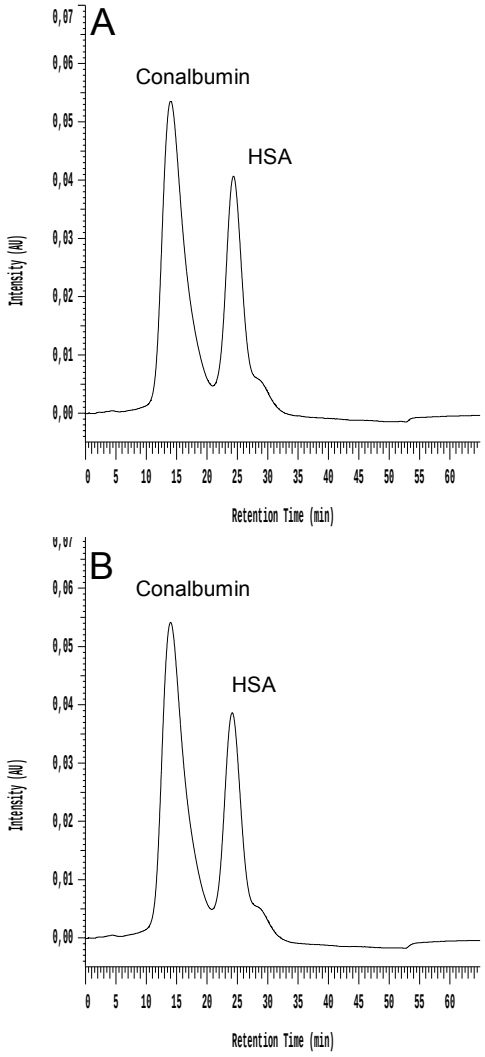


Fig 4. Representative UV-traces of the separation of a mixture of conalbumin and HSA on Fractogel® TMAE scout columns (**A**) or laboratory scale columns (**B**, dimensions: 100 x 16 mm). For details see text.

Fractogel® EMD TMAE Hicap (M)

Using Fractogel® TMAE Hicap scout columns, conalbumin and HSA were separated as documented in figure 5A. The representative UV trace for three independent experiments is shown. As a comparison, the same proteins were separated under identical conditions using a laboratory-scale column packed with Fractogel® EMD TMAE Hicap (M) (dimension 100 x 16 mm), however sample load was increased 20-fold to maintain the scale. A representative UV-trace of this separation is shown in Fig 5 B.

Resolution factors for the separation of conalbumin and HSA were as follows: 2.05 for scout columns and 2.1 for laboratory-scale columns. The data indicates that, using the 1 ml- Fractogel® TMAE scout columns for model protein mixtures, calculated resolution remains appromimately equal as compared to the resolution of a 100 x 16 mm-laboratory-scale column.

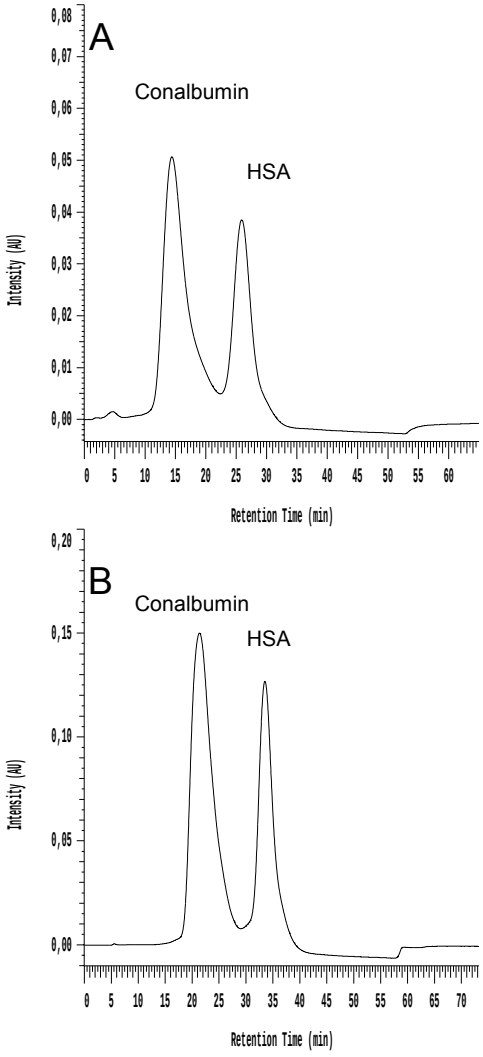


Fig 5. Representative UV-traces of the separation of a mixture of conalbumin and HSA on Fractogel® TMAE Hicap scout columns (**A**) or laboratory scale columns (**B**, dimensions: 100 x 16 mm). For details see text.

Elution conductivities in mS/cm on Fractogel® EMD TMAE Hicap (M) for the respective model proteins are summarized in table 6. The elution conductivities for the two tested model proteins on Fractogel® TMAE Hicap scout columns were reproducibly found to be approximately 10 % lower as compared to the

laboratory-scale columns (dimension 100 x 16 mm) in case of conalbumin and 14 % lower in case of HSA, respectively. Thus elution conductivity of a given target biomolecule on a laboratory-scale column (100 x 16 mm) can be predicted to be ~ 12 % higher as in case of the scout column provided the described experimental setup is maintained.

Table 6. Elution conductivities of model proteins on Fractogel® EMD TMAE Hicap (M)

Protein	Fractogel® TMAE Hicap scout column	Fractogel® EMD TMAE (M) column (100 x 16 mm)
Conalbumin	11.6 ± 0.7	13.5 ± 0.1
Human Serum Albumin	22.4 ± 0.7	24.8 ± 0.8

7. Screening for experimental conditions using Fractogel® scout columns

To allow for rapid screening of experimental parameters in biomolecule purification, Fractogel® scout columns have been designed for using higher flow rates up to 600 cm/hour (5.2 ml/min). However, please acknowledge that resolution factors for a given separation will decrease with elevated flow rates.

As a guideline to estimate the effect of increased flow rates for a given protein separation, please find below a pressure-flow curve and data on the relation between resolution factors for Fractogel® TMAE scout columns.

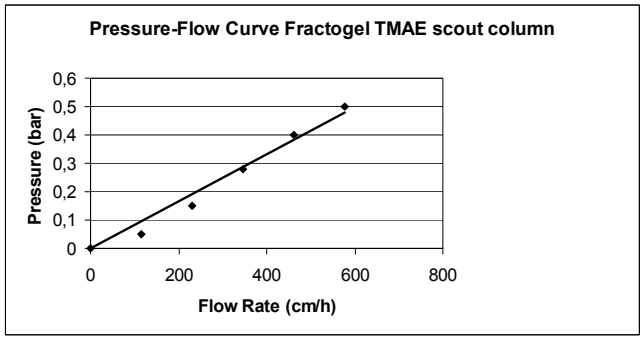


Fig. 6 Pressure-flow curves for Fractogel® TMAE scout columns. Pressure drops were reproducibly found to be as low as 0.5 bar at flow rates of 580 cm/hour.

Fig. 6 shows a representative pressure-flow curve for Fractogel® TMAE scout columns. At a linear flow rate of 580 cm/hour, the measured pressure drop of the columns has been as low as 0.5 bar. Thus rapid screening experiments for scouting of biomolecule purification parameters are feasible using Fractogel® scout columns.

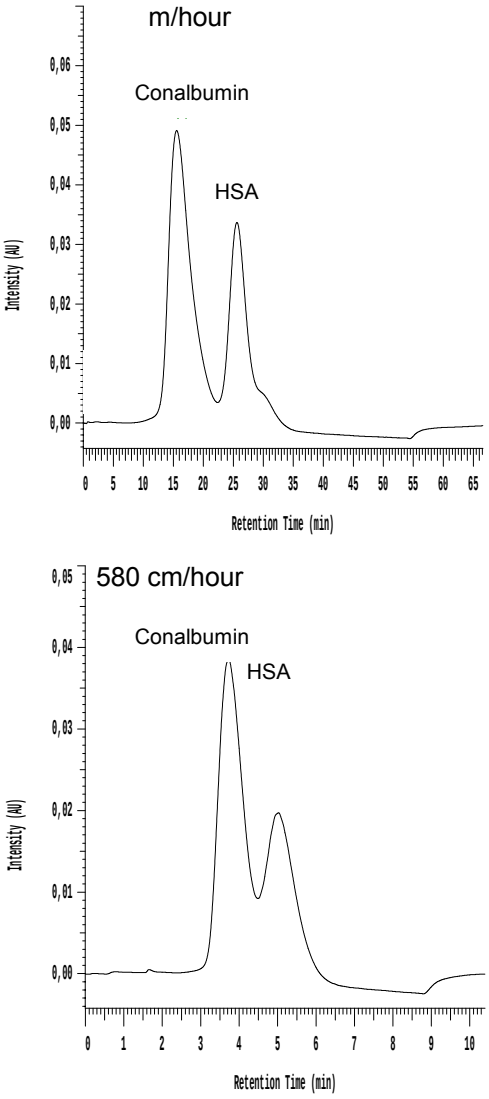


Fig. 7 Representative UV-traces of the separation of a mixture of Conalbumin and Humas Serum Albumin on Fractogel TMAE scout columns at linear flow rates of 78 cm/hour or 580 cm/hour.

To determine the effect of increased flow rate on resolution, the model protein mixture described in chapter 6 has been separated on Fractogel® TMAE scout columns at linear flow rates of 78 cm/hour or 580 cm/hour respectively.

Figure 7 shows representative UV traces of these separations. Resolution factors for the respective separation dropped from 1.85 in case of a flow rate of 78 cm/hour to 0.9 in case of a flow rate of 580 cm/hour. Thus, resolution clearly decreases with increased flow rate, however separation of the described model protein mixture on Fractogel® TMAE scout columns remains possible at 580 cm/hour.

NOTE:
If maximum possible resolution is the main goal of scouting experiments, operating flow rates should not exceed 150 cm/hour.

The Fractogel® scout columns have been tested for a total of 100 runs including a Cleaning-in-place step for the separation of model proteins using the following conditions:

Column:	Fractogel® TMAE scout column
Sample:	100 µl 5 mg/ml each protein (Conalbumin and HSA)
Detection:	280 nm
Solutions:	**A**: 20 mM Tris/HCl buffer, pH 8.0 (1.2 mS/cm) **B**: 20 mM Tris/HCl buffer, 1 M NaCl, pH 8.0; (85.8 mS/cm) **C**: 1 M NaOH in water

Gradient:

0 min/0 % B	0.67 ml/min
3.0 min/0 % B	0.67 ml/min (2 CV buffer A)
3.1 min/0 % B	2.0 ml/min
19.9 min/50 % B	2.0 ml/min
20.0 min/100 % B	2.0 ml/min
22.0 min /100 % B	2.0 ml/min (4 CV buffer B)
22.1 min / 100%C	0.25 ml/min
42.1 min / 100% C	0.25 ml/min (5 CV 1 M NaOH)
42.2 min / 100% B	2.0 ml/min
44.2 min / 100% B	2.0 ml/min (4 CV buffer B)
44.3 min / 100% A	2.0 ml/min
51.3 min/ 100 % A	2.0 ml/min (14 CV buffer A)

Temperature: room temperature

The required volume of buffer A for re-equilibration at the end of the method was checked. 14 column volumes were necessary to achieve both conductivity and pH equilibration. Please acknowledge that the operating flow rate should be lowered to 0.25 ml/min for Cleaning-in-place using 1 M NaOH in water due to the higher viscosity of that solution.
Under these conditions the UV traces of runs number one and number one hundred on a given Fractogel® TMAE scout column were found to be undistinguishable indicating the column to be stable for these number of runs under the applied conditions (data not shown). However, please acknowledge that the column lifetime will be strongly depending on the applied sample and on the respective buffers used. Thus the total number of runs that can be performed with one column may be lower in case of customer-specific conditions.

Fractogel® AEX screening kit

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8. Biological buffers

It is of critical importance to use the highest-purity water available for preparation of biological buffers for biomolecule purification.

Always try to prepare buffers at the temperature and concentration that you plan to use during your experiement. When working with stock solutions, always adjust the pH after dilution and temperature adjustment.

When selecting an appropriate buffer substance, always choose one with a pK_α close to the desired working pH. For buffer pK_α-values please refer to table 7. For buffering range of biological buffers please refer to figure 8.

NOTE:
For cation exchange chromatography, always use a buffer substance that will have a negative net charge at the working pH. For anion exchange chromatography, please use a buffer substance that will have a positive net charge at the working pH.

Buffer	pK_α (4 °C)	pK_α (20 °C)	pK_α (25 °C)	pK_α (37 °C)	ΔpK_α/°C
ACES	7.22	6.90	6.80	6.56	−0.020
ADA	6.80	6.62	6.56	6.43	−0.011
BES	7.41	7.15	7.07	6.88	−0.016
BICINE	8.64	8.35	8.26	8.04	−0.018
BIS-TRIS	6.88	6.56	6.46	6.22	−0.020
CHES	9.73	9.55	9.50	9.36	−0.011
Citrate $pK_{\alpha 2}$	4.79	4.77	4.76	4.74	−0.0016
Glycine $pK_{\alpha 2}$	10.32	9.91	9.78	9.47	−0.026
Gly-Gly	8.85	8.40	8.26	7.92	−0.028
HEPES	7.77	7.55	7.48	7.3	−0.014
HEPPS	8.18	8.00	7.95	7.8	−0.011
Imidazole	7.37	7.05	6.95	6.71	−0.020
MES	6.33	6.15	6.10	5.97	−0.011
MOPS	7.41	7.20	7.14	6.98	−0.013
PIPES	6.94	6.80	6.76	6.66	−0.0085
Phosphate $pK_{\alpha 2}$	7.26	7.21	7.20	7.17	−0.0028
TAPS	8.02	8.31	8.40	8.62	+0.018
TES	7.82	7.50	7.40	7.16	−0.020
TRICINE	8.49	8.15	8.05	7.79	−0.021
TRIS	8.75	8.30	8.08	7.82	−0.028

Table 7. pK_α -values of selected biological buffers

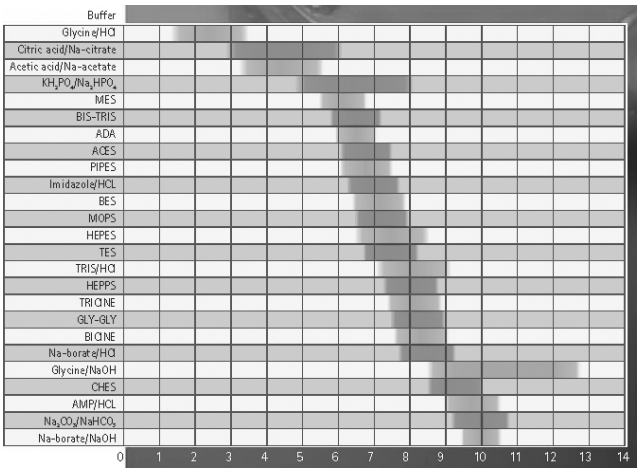

Fig 8. Buffering ranges of selected biological buffers

Fractogel® AEX screening kit

Cat. No. 1.50502.0001

Fractogel® EMD tentacle media / Ordering Information

Designation	Cat.-No.	Particle Size	Content
Chromatography type: strong anion exchanger			
Fractogel® EMD TMAE (M)	1.16881.0010 1.16881.0100 1.16881.0500 1.16881.5000	40 – 90 µm	10 ml 100 ml 500 ml 5000 ml
Fractogel® EMD TMAE Hicap (M)	1.10316.0010 1.10316.0100 1.10316.0500 1.10316.1000	40 – 90 µm	10 ml 100 ml 500 ml 5000 ml
Fractogel® EMD TMAE (S)	1.16887.0010 1.16887.0100 1.16887.0500	20 – 40 µm	10 ml 100 ml 500 ml
Fractoprep® TMAE	1.17973.0010 1.17973.0100 1.17973.1000 1.17973.5000	30 – 150 µm	10 ml 100 ml 1000 ml 5000 ml
Designation	**Cat.-No.**	**Particle Size**	**Content**
Chromatography type: weak anion exchanger			
Fractogel® EMD DEAE (M)	1.16883.0010 1.16883.0100 1.16883.0500 1.16883.5000	40 – 90 µm	10 ml 100 ml 500 ml 5000 ml
Fractogel® EMD DEAE (S)	1.16888.0010 1.16888.0100 1.16888.0500	20 – 40 µm	10 ml 100 ml 500 ml
Fractogel® EMD DMAE (M)	1.16884.0010 1.16884.0100 1.16884.0500 1.16883.5000	40 – 90 µm	10 ml 100 ml 500 ml 5000 ml
Fractogel® EMD DMAE (S)	1.16887.0010 1.16887.0100 1.16887.0500	20 – 40 µm	10 ml 100 ml 500 ml
Fractoprep® DEAE	1.17971.0010 1.17971.0100 1.17971.1000 1.17971.5000	30 – 150 µm	10 ml 100 ml 1000 ml 5000 ml
Designation	**Cat.-No.**	**Particle Size**	**Content**
Chromatography type: strong cation exchanger			
Fractogel® EMD SO_3^- (M)	1.16882.0010 1.16882.0100 1.16882.0500 1.16882.5000	40 – 90 µm	10 ml 100 ml 500 ml 5000 ml
Fractogel® EMD SO_3^- (S)	1.16890.0010 1.16890.0100 1.16890.0500	20 – 40 µm	10 ml 100 ml 500 ml
Fractogel® EMD SE Hicap (M)	1.14894.0010 1.14894.0100 1.14894.0500 1.14894.5000	40 – 90 µm	10 ml 100 ml 500 m 5000 ml
Fractoprep® SO_3^-	1.17972.0010 1.17972.0100 1.17972.1000 1.17972.5000	30 – 150 µm	10 ml 100 ml 1000 ml 5000 ml
Designation	**Cat.-No.**	**Particle Size**	**Content**
Chromatography type: weak cation exchanger			
Fractogel® EMD COO^- (M)	1.16886.0010 1.16886.0100 1.16886.0500 1.16886.5000	40 – 90 µm	10 ml 100 ml 500 ml 5000 ml
Fractogel® EMD COO^- (S)	1.16891.0010 1.16891.0100 1.16891.0500	20 – 40 µm	10 ml 100 ml 500 ml