KNAUER

Ion-exchange chromatography (IEC)

separation according to charge

BioFox 17 Q and 40 Q

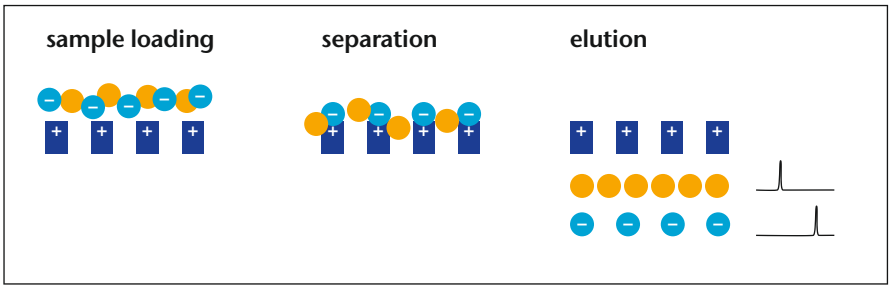
Anion-exchange chromatography

Analytical and preparative separations of proteins

Ion-exchange chromatography separates molecules according to type and strength of their charge. For this, a column is used which consists of separation media beads that are either positively or negatively charged.

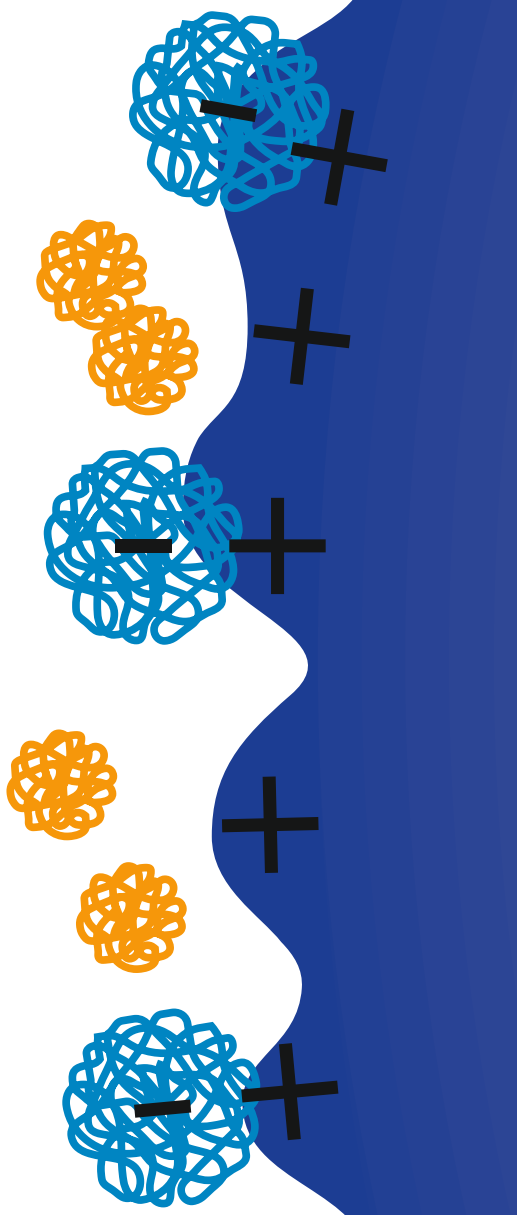
A positively charged bead, known as an anionic-exchanger, will tend to bind to biomolecules with a net negative charge, and a negatively charged bead, known as a cationic-exchanger, will tend to bind to biomolecules with a net positive charge. The binding of the biomolecules to the beads is fully reversible and their removal (elution) is usually achieved through the flow of increasing amounts of sodium chloride salt down the column.

The sodium or chloride ions compete with the binding of the biomolecules to the charged beads causing the biomolecules to be released and allowing them to be eluted out of the bottom of the column. The order in which the biomolecules are eluted is dependant upon their net charge, with the weakest charged coming off first.



The complex surface of biomolecules consists mostly of both anions and cations whose charge is just neutralized at the isoelectric point (pH=IP). By carefully choosing the eluent's pH value, a suitable range above the IP (exchange of anions with quaternary ammonium groups =Q) or below the IP (exchange of cations with sulfonic acid groups=S) can be determined in order to separate target molecules.

Ion-exchange chromatography is a technique with very high binding capacities, high flow characteristics and potentially excellent resolution. It is therefore perfect for the separation of large volumes of sample (fluid feed) and fits well into the early or capture step of a purification methodology.



Pressure stability up to 40 bar (580 psi) – fast and high resolution biochromatography

BioFox ion-exchange media are produced from agarose beads using a proprietary cross-linking method that results in a highly porous and physically stable agarose matrix. Besides the well-known selectivity of agarose, these media are pressure resistant 40 bar (580 psi) for high resolution biochromatography. Two different particle sizes are available for analytical and preparative anion-exchange: BioFox 17 Q and BioFox 40 Q.

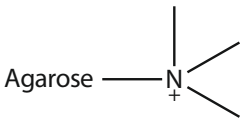
BioFox 17 Q

- Made from agarose, well established and well-known in the biotech industry
- Outstanding resolution even at high protein loads due to a highly selective 17 µm anion-exchange media
- Robust separation results can be achieved across a wide range of proteins and separation conditions
- Ready for immediate use with Bioline instruments and most other chromatography systems

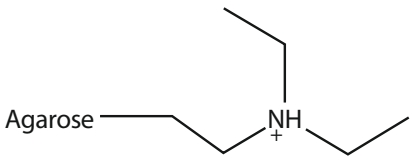
BioFox 40 Q

- Made from agarose, well established in the biotech industry
- High throughput and resolution
- Reliable and reproducible
- High chemical stability for easy cleaning-in-place (CIP)
- Easy and reliable scale-up

a)



b)



a) Q anion-exchanger: strong
b) DEAE (diethylaminoethyl) anion-exchanger: weak

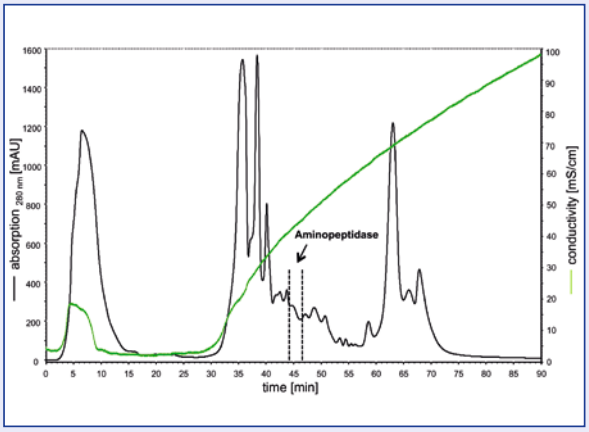
The Bioline Rack – with glass columns

BioFox 17 Q

BioFox 17 Q anion-exchange medium is based on a small particle size of 17 µm with a very narrow size distribution. In combination with the proprietary cross-linking, this small bead size results in columns with optimal efficiency and good flow characteristics.

BioFox 17 Q is designed for high performance protein separations under ion-exchange conditions. The high resolution that can be obtained makes this chromatography media ideal for both demanding quantitative analysis and semi-preparative work.

Extraction of proteins from *Lactobacillus sp.*



Separation column
BioFox 17 Q, 8 x 85 mm (4.3 ml)

Separation conditions

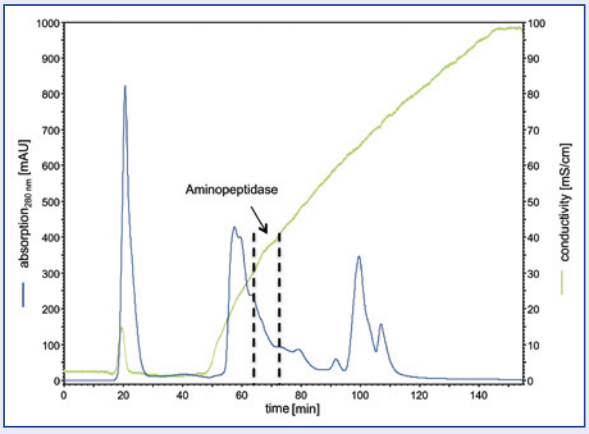
Sample:	12.5 ml crude extract from bacteria
Flow rate:	1.5 ml/min
Buffer:	A 10 mM Tris-HCl pH 7.5, B 10 mM Tris-HCl pH 7.5 + 1.0 M NaCl
Detection:	(UV) 280 nm

BioFox 40 Q

BioFox 40 Q anion-exchange medium has a particle size of 40 µm with a very narrow particle size distribution. This results in high column efficiency with excellent flow characteristics suited to demanding bioprocess applications.

BioFox 40 Q is designed for high throughput protein separations under ion-exchange conditions. Since anion-exchange capacity is high, BioFox 40 Q has the capacity to separate proteins satisfactorily even when using high protein loadings. In combination with high flow rates, BioFox 40 Q is ideal for process applications. The chemical stability means it is easy to develop cleaning-in-place (CIP) protocols using sodium hydroxide.

Extraction of proteins from *Lactobacillus sp.*



Separation media
BioFox 40 Q

Separation conditions

Sample:	12.5 ml crude extract from bacteria
Flow rate:	1.5 ml/min
Buffer:	A 20 mM Tris-HCl pH 7.0, B 20 mM Tris-HCl pH 7.0 + 1.0 M NaCl
Detection:	(UV) 280 nm

Courtesy of T. Eisele and T. Stressler (Working group of Prof. Fischer)
Universität Hohenheim, Germany

Media characteristics	BioFox 17 Q	BioFox 40 Q	DEAE
Particle size [µm]	16–18	32–60	
Agarose content [%]	7.4– 7.8		7.5– 7.8
Protein capacity	Test protein BSA, 130 mg/ml		mg BSA/ml at 60 cm/h bed height, 85 mg/ml
Ionic group	Quatenary amine		Di-ethylaminoethyl
Ionic capacity [mmol/ml]	0.18–0.26		
Max flow rate at 20 cm bed height [cm/h]	> 500		
pH stability	1–14		
Solvent stability	100% methanol, 100% ethanol, 8 M urea, 6 M guanidine hydrochloride, 30% acetonitrile, 70% formic acid, 30% trifluoroacetic acid		

Column specifications	BioFox 17 Q	BioFox 40 Q
Optimal operating flow rate [ml/min]	0.5–2.0	0.5–5.0
Maximum operating flow rate [ml/min]	4	10
Mesh size of the net [µm]	10	
pH stability	1–14	
Operating temperature [°C]	4–40	
Cleaning	Columns can be sanitized with 0.5 M NaOH or 70% ethanol.	
Materials in contact with eluent	PEEK (polyether ether ketone) (tubing), EPDM (O-ring), PVDF (polyvinyldifluoride) (adaptor).	
Solvent resistance	Methanol, ethanol, 8 M urea, 6 M guanidinium hydrochloride, 30% acetonitrile, 70% formic acid, 1 M sodium hydroxide, 0.1 M hydrochloride acid, 5% sodium do-decyl sulphate, 5% 2-mercaptoethanol, 30% acetic acid, 0.1% trifluoroacetic acid.	

Available sizes (pre-packed columns)	BioFox 17 Q	BioFox 40 Q
	ion-exchange agarose media – pre-packed in analytical grade columns – preserved with 22% ethanol	
Media volume [ml]	4.3	
Internal diameter [mm]	8	
Bed height [mm]	85	

Ordering information BioFox 17 Q	
Order No.	**Column size/volume**
Y4008	Pre-packed column, 8x85 mm, 4.3 ml
Ordering information BioFox DEAE	
Order No.	**Column size/volume**
Y4026	Bulk media, 1 l
Y4031	Bulk media, 25 ml

Ordering information BioFox 17 Q	
Order No.	**Column size/volume**
Y4019	Pre-packed column, 8x85 mm, 4.3 ml
Y4011	Bulk media, 25 ml
Y4022	Bulk media, 200 ml
Y4002	Bulk media, 1 l
Y4082	Bulk media, 5 l

Technical data are subject to change without notice. Please check our website for latest updates and changes.

Visit www.knauer.net for more information on KNAUER's complete range of biochromatography products.

HPLC · SMB · Osmometry

Wissenschaftliche Gerätebau
Dr. Ing. Herbert Knauer GmbH
Hegauer Weg 38
14163 Berlin, Germany

Phone: +49-(0)30-809727-0
Telefax: +49-(0)30-8015010
E-Mail: info@knauer.net
Internet: www.knauer.net