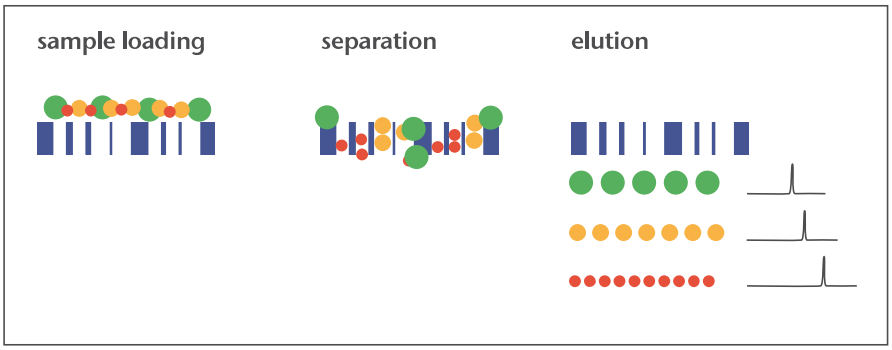
Size exclusion chromatography (SEC)

separation according to size

▶ BioFox 17 SEC and 40 SEC

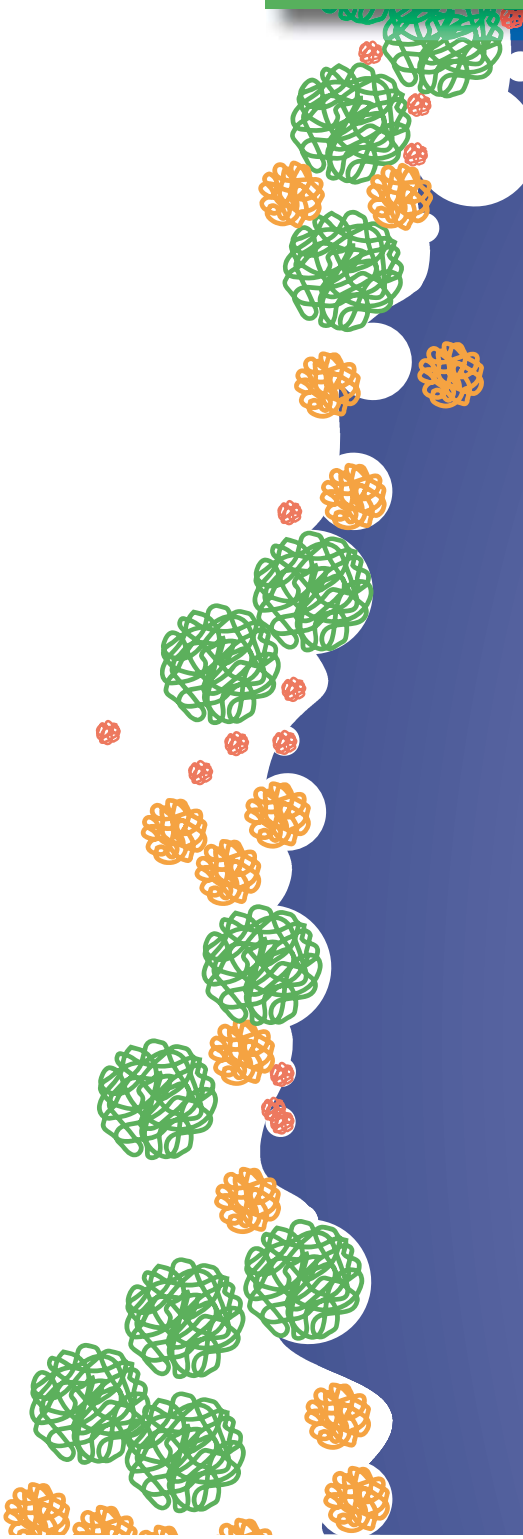
Analytical and preparative separations of proteins

Size exclusion chromatography separates molecules according to their different sizes. This technique is used in the intermediate and last stage polishing of almost all bioseparation protocols.



For decades, agarose-based supports have been successfully used in these protocols for biotechnology research and industrial scale protein purification. Agarose is proven to be exceptionally compatible with naturally ocurring bio molecules, like e. g. proteins, DNA and carbohydrates.

The packing material shows only negligible non-specific interactions due to the hydrophilic nature of agarose and allows the use of non-denaturating mobile phases. Unlike media from synthetic polymers, agarose does not contain micropores. This prevents local pH fluctuations within the column that would worsen the separation.



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

BioFox SEC 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 up to 40 bar (580 psi) for high resolution biochromatography. Two different particle sizes are available for analytical and preparative purposes: BioFox 17 SEC and BioFox 40 SEC.

BioFox 17 SEC

- Made from agarose, well-established in the biotech industry
- Outstanding resolution
- Robust separation results can be achieved across a wide range of proteins and separation conditions
- Ready for immediate use with Bioline and in most chromatography systems in the market

BioFox 40 SEC

- Made from agarose, well-established in the biotech industry
- Excellent resolution at preparative scale
- Robust separation results across a wide range of proteins and conditions
- Chemically stable for cleaning-in-place (CIP) procedures

The Bio
with gl

Significantly save time and improve the performance of your bioseparations!

Packing technique	Standard procedure	KNAUER high-pressure packing
Packing pressure	Atmospheric pressure	15 bar (218 psi)
Duration of packing procedure[1]	12 h	2 h
Separation performance		
Theoretical plates (ASTM)[2]	2138	5003

1) BioFox 40/1200 SEC material, Bioline HR glass column, 20 mm ID x 60 cm length

2) determined with acetone test

Up to 3x higher resolution
Up to 3x more throughput

KNAUER

BioFox 17 SEC filtration gels

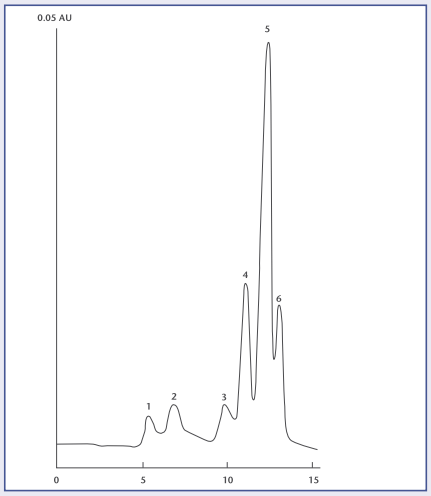
The BioFox 17 SEC material for gel filtration has a higher selectivity for proteins than most stationary phases made from synthetic polymers. Consequently these SEC media have the capacity to successfully separate proteins, even when loading high amounts of protein. The small particle size of 17 µm and the narrow size distribution in combination with the proprietary cross-linking results in column packings with optimal efficiency and good flow characteristics.

Resolution is the combined effect of selectivity (distance between peaks) and efficiency (peak width, depending on particle size). BioFox 17 SEC was developed for high performance protein separations under varying conditions. Due to the high resolution, sharp and well-separated peaks can be achieved, which makes these media ideal for analytical and semi-preparative FPLC applications.

BioFox 40 SEC filtration gels

Featuring a particle size distribution of 40 µm BioFox 40 SEC gels are optimized for preparative scale separations. Special cross-linking increases the pressure stability of the gel, allowing higher flow rates and much faster column packing with KNAUER Bioline glass columns. Excellent column bed quality results in high efficiency and good flow characteristics. The high resolution that can be achieved makes BioFox 40 SEC ideal for both lab scale preparative work and process scale separations of proteins.

Separation of molecular weight standards on BioFox 17/1200 SEC

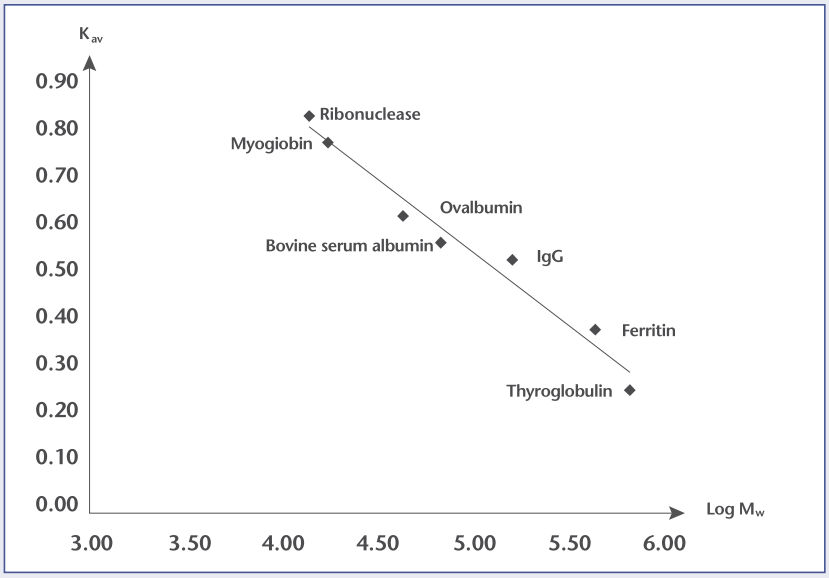


Separation column
pre-packed BioFox 17/1200 column, 300 x 8 mm ID, 15 ml

Separation conditions
Flow rate: 0.1 ml/min
Buffer: 0.05 M sodium phosphate + 0.15 M NaCl pH 6.7

1	Thyroglobulin	669 kDa
2	Aldolase	158 kDa
3	Ovalbumin	43 kDa
4	Carbonic anhydr.	29 kDa
5	Myoglobin	16.7 kDa
6	Ribonuclease	13.7 kDa

High selectivity of BioFox 40/1200



Partition coefficient K_{av} plotted over the logarithm of molecular weight for selected proteins. The selectivity curve is straight over a range from K_{av}=0.2 to K_{av}=0.8. Here, the dimer of thyroglobulin elutes in the void volume V_0 (K_{av} = 0) and the other proteins nicely follow the theoretical K_{av} curve.

line Rack –
ass columns

Media characteristics	BioFox 17/1200 SEC	BioFox 17/150 SEC	BioFox 40/1200 SEC	BioFox 40/150 SEC	BioFox 40/10000 SEC
Exclusion limit	1200 kDa	150 kDa	1200 kDa	150 kDa	10000 kDa
Max flow rate in an 8 x 300 mm column	–		15 cm/min		10 cm/min
Particle size	16 – 18 µm		32 – 60 µm		
Agarose content	7.4 – 7.8 %	9.2 – 9.5 %	7.4 – 7.8 %	9.2 – 9.8 %	4.6-5.0 %
Pressure stability (media)	40 bar				
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 17/1200 SEC	BioFox 17/150 SEC	BioFox 40/1200 SEC	BioFox 40/150 SEC	BioFox 40/10000 SEC
Plates/m	25 000 – 30 000		8 000 – 11 000		
Mesh size of the net	10 µm				
Optimal operating flow rate	0.5 – 2.0 ml/min				
Maximum operating flow rate	3 ml/min for 300 mm columns, 1.8 ml/min for 500 mm columns				
pH stability	1 – 14				
Pressure stability (pre-packed column)	20 bar				
Asymmetry	0.85 – 1.15				
Operating temperature	4 – 40 °C				
Cleaning	Columns can be sanitized with 0,5 M NaOH or 70 % ethanol.				
Materials in contact with eluent	Borosilicate glass (chromatographie tube), titanium (filter), PEEK (polyetheretherketone) (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.				

BioFox SEC media are supplied as an aqueous suspension with 22 % ethanol as a preservative. After washing the media are immediately ready for use.

Ordering information pre-packed column	
Order No.	**Column size/volume**
30GX46KBFW	BioFox SEC 17/1200, 300 x 8 mm ID, 15 ml
50GX46KBFW	BioFox SEC 17/1200, 500 x 8 mm ID, 25 ml
30GX46JBFW	BioFox SEC 17/150, 300 x 8 mm ID, 15 ml
30GX46KBFZ	BioFox SEC 40/1200, 300 x 8 mm ID, 15 ml
30GX46JBFZ	BioFox SEC 40/150, 300 x 8 mm ID, 15 ml

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

Ordering information bulk media	
Order No.	**Column size/volume**
00OX46KBFZ	BioFox 40/1200 SEC, 300 ml
00QX46KBFZ	BioFox 40/1200 SEC, 1 l
00RX46KBFZ	BioFox 40/1200 SEC, 5 l
00OX46JBFZ	BioFox 40/150 SEC, 300 ml
00QX46JBFZ	BioFox 40/150 SEC, 1 l
00OX46LBFZ	BioFox 40/10000 SEC, 300 ml
00QX46LBFZ	BioFox 40/10000 SEC, 1 l

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

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14163 Berlin, Germany

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Internet: www.knauer.net

Your local distributor:

Ion-exchange chromatography (IEC)

separation according to charge

BioFox 17/1200 Q, 40/1200 Q and 40/1200 DEAE

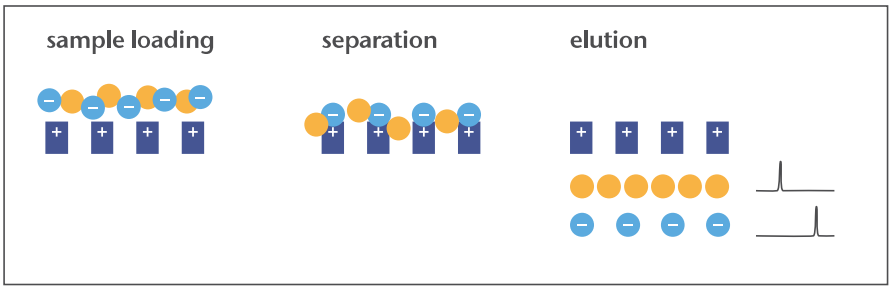
Anion-exchange chromatography

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 a strong exchanger/ Q= quaternary ammonium group or a weak exchanger/ DEAE=di-ethylaminoethyl group) 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 resistant up to 40 bar (580 psi) for high resolution biochromatography. Two different particle sizes are available for analytical and preparative anion-exchange: BioFox 17/1200 and BioFox 40/1200.

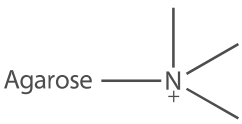
BioFox 17/1200

- 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

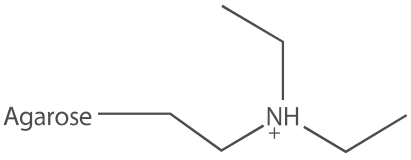
BioFox 40/1200

- 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

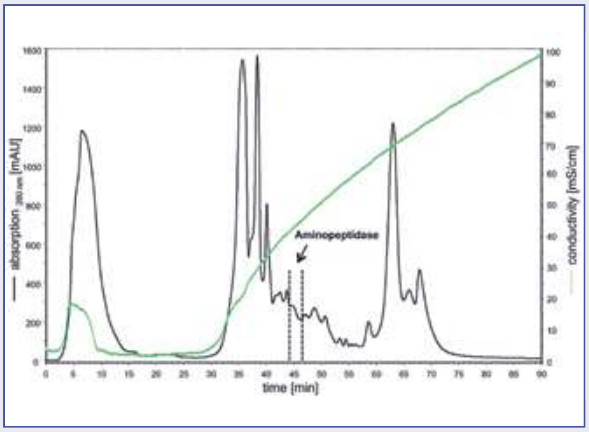
KNAUER

BioFox 17/1200

BioFox 17/1200 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 17/1200 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 qualitative analysis and semi-preparative work.

Extraction of proteins from *Lactobacillus sp.*



Separation column
pre-packed BioFox 17/1200 Q, 85 x 8 mm ID (4,3 ml)

Separation conditions

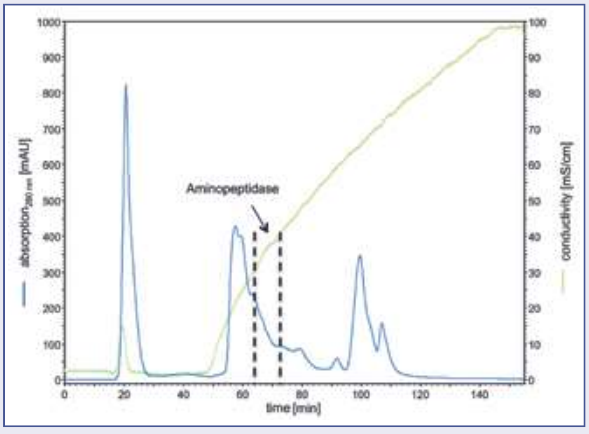
Sample:	2.5 ml crude extract from bacteria
Flow rate:	1.5 ml/min
Buffer:	A 10 mM Tris-HCl pH 7.5, B 10 mM Tris-HCl pH 7.5 + 1.0 M NaCl
Detection:	(UV) 280 nm

BioFox 40/1200

BioFox 40/1200 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 40/1200 is designed for high throughput protein separations under ion-exchange conditions.
Since anion-exchange capacity is high, BioFox 40/1200 has the capacity to separate proteins satisfactorily even when using high protein loadings. In combination with high flow rates, BioFox 40/1200 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 40/1200 Q

Separation conditions

Sample:	2.5 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 17/1200 Q	BioFox 40/1200 Q	BioFox 40/1200 DEAE
Particle size	16–18 µm	32–60 µm	
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	0.18–0.26 mmol/ml		
Max flow rate at 20 cm bed height	> 500 cm/h		
Pressure stability (media)	40 bar		
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 17/1200 Q	BioFox 40/1200 Q
Optimal operating flow rate	0.5–2.0 ml/min	0.5–2.0 ml/min
Maximum operating flow rate	2 ml/min	
Mesh size of the net	10 µm	
Pressure stability (pre-packed column)	10 bar	
pH stability	1–14	
Operating temperature	4–40 °C	
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.	

BioFox ion exchange media are supplied in aqueous suspensions with 22 % ethanol as preservative and are immediately ready for use after washing.

Ordering information BioFox 17/1200 Q	
Order No.	**Column size/volume**
08GX15HBFW	pre-packed column 85 x 8 mm ID, 4.3 ml

Ordering information BioFox 40/1200 DEAE	
Order No.	**Column size/volume**
00IX15IBFZ	Bulk media, 25 ml
00QX15IBFZ	Bulk media, 1 l
00RX15IBFZ	Bulk media, 5 l

Ordering information BioFox 40/1200 Q	
Order No.	**Column size/volume**
08GX15HBFZ	Pre-packed column, 8 x 85 mm, 4.3 ml
00IX15HBFZ	Bulk media, 25 ml
00MX15HBFZ	Bulk media, 200 ml
00QX15HBFZ	Bulk media, 1 l
00RX15HBFZ	Bulk media, 5 l

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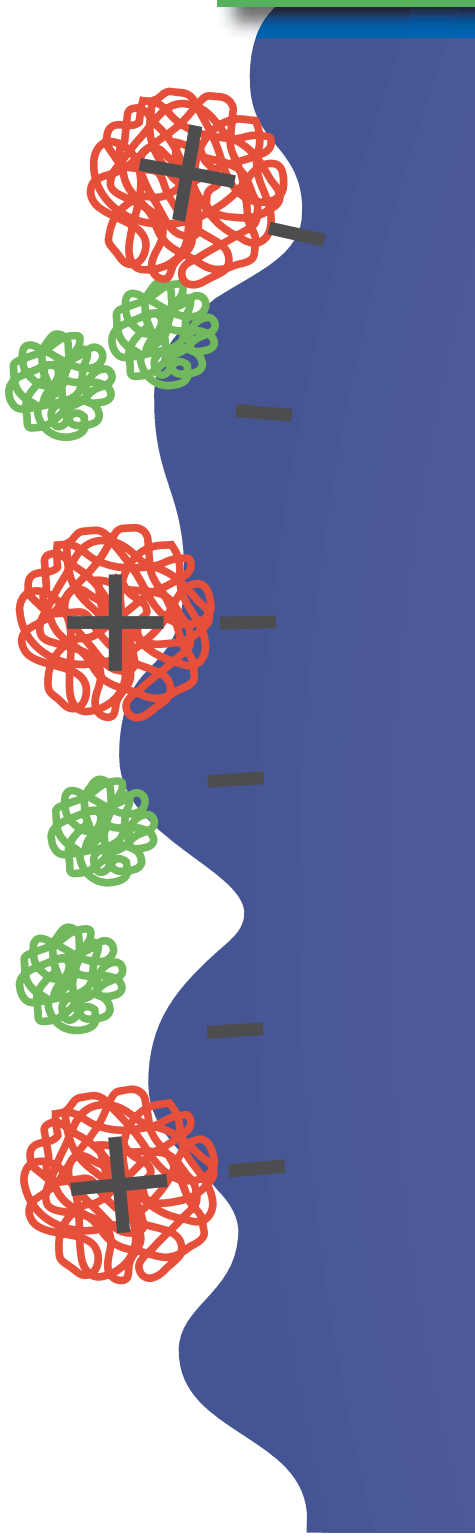
Ion-exchange chromatography (IEC)

separation according to charge

BioFox 17/1200 S and BioFox 40/1200 S

Cation-exchange chromatography

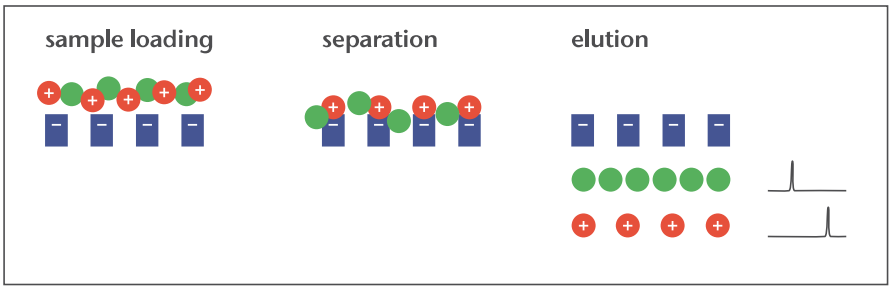


Analytical and preparative separations of proteins

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 dependent 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 pH value of the eluent, 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

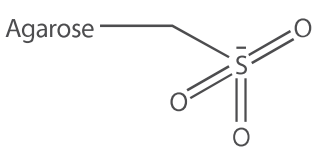
BioFox ion-exchange media are produced from agarose beads using a proprietary cross-linking method that results in a highly porous and physically stable agarose matrix. Besides the well-known selectivity of agarose, these media are pressure resistant up to 40 bar (580 psi) for high resolution biochromatography. Two different particle sizes are available for analytical and preparative cation-exchange: BioFox 17/1200 S and BioFox 40/1200 S.

BioFox 17/1200 S

- 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 cation-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 40/1200 S

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



S cation-exchanger: strong

The Bioline Rack – with glass columns

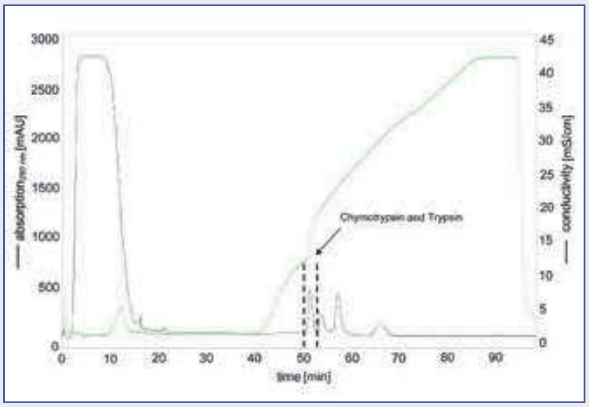
KNAUER

BioFox 17/1200 S

BioFox 17/1200 S cation-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 packed columns with very high efficiency and good flow characteristics.

BioFox 17/1200 S is designed for high performance protein separations under ion-exchange conditions. The high resolution that can be obtained makes this ion-exchange media ideal for both demanding quantitative analysis and semi-preparative work.

Extraction of proteins from *Lactobacillus sp.*



Separation column
pre-packed BioFox 17/1200 S 85 x 8 mm ID (4,3 ml)

Separation conditions

Sample:	5 ml of bovine pancreatic extract, pretreated with acid precipitation and heavy metal precipitation to remove contaminating proteins
Flow rate:	0.2 ml/min
Buffer:	A 20 mM Tris pH 7.0, B 20 mM Tris + 0.5 M sodium acetate
Detection:	(UV) 280 nm

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

BioFox 40/1200 S

BioFox 40/1200 S cation-exchange medium has a particle size of 40 µm with a narrow particle size distribution. This results in high column efficiency with optimal flow characteristics suitable for demanding bioprocess applications.

BioFox 40/1200 S is designed for high throughput protein separations under ion-exchange conditions. Since cation-exchange capacity is high, BioFox 40/1200 S has the capacity to separate proteins satisfactorily even when using high protein loadings. In combination with high flow rates, it is ideal for process applications. Furthermore, this medium is chemically stable which makes for easy development of cleaning-in-place (CIP) protocols using sodium hydroxide.

Media characteristics	BioFox 17/1200 S	BioFox 40/1200 S
Particle size	16–18 µm	32–60 µm
Ionic group	Sulfonic acid	
Ionic capacity	0.18–0.26 mmol/ml	
Max flow rate at 20 cm bed height	> 500 cm/h	
Pressure stability (media)	40 bar	
Agarose content	7.4 – 7.8 %	
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	
Protein capacity	Test protein IgG 70 mg/ml	

Column specifications	BioFox 17/1200 S	BioFox 40/1200 S
Optimal operating flow rate	0.5 – 2.0 ml/min	0.5 – 2.0 ml/min
Maximum operating flow rate	2 ml/min	
Pressure stability (pre-packed column)	10 bar	
Mesh size of the net	10 µm	
pH stability	1–14	
Operating temperature	4–40 °C	
Cleaning	Columns can be sanitized with 0.5 M NaOH or 70 % ethanol.	
Materials in contact with eluent	PEEK (poly ether etherketone) (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 dodecyl sulphate, 5 % 2-mercaptoethanol, 30 % acetic acid, 0.1 % trifluoroacetic acid.	

BioFox ion exchange media are supplied in aqueous suspensions with 22 % ethanol as preservative and are immediately ready for use after washing.

Ordering information pre-packed column	
Order No.	**Volume**
08GX14GBFZ	BioFox 40/1200 S column, 85 x 8 mm ID, 4.3 ml
08GX14GBFW	BioFox 17/1200 S column, 85 x 8 mm ID, 4.3 ml

Ordering information BioFox 40/1200 S	
Order No.	**Volume**
00IX14GBFZ	BioFox 40/1200, 25 ml
00MX14GBFZ	BioFox 40/1200, 200 ml
00QX14GBFZ	Biofox 40/1200, 1 l
00RX14GBFZ	Biofox 40/1200, 5 l

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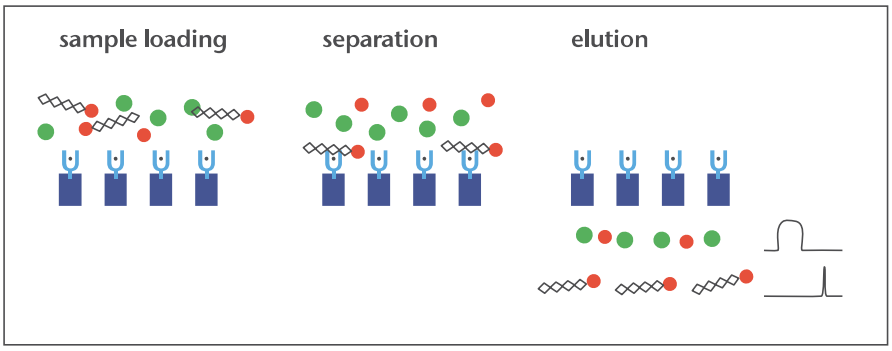
Immobilized metal ion affinity chromatography (IMAC)

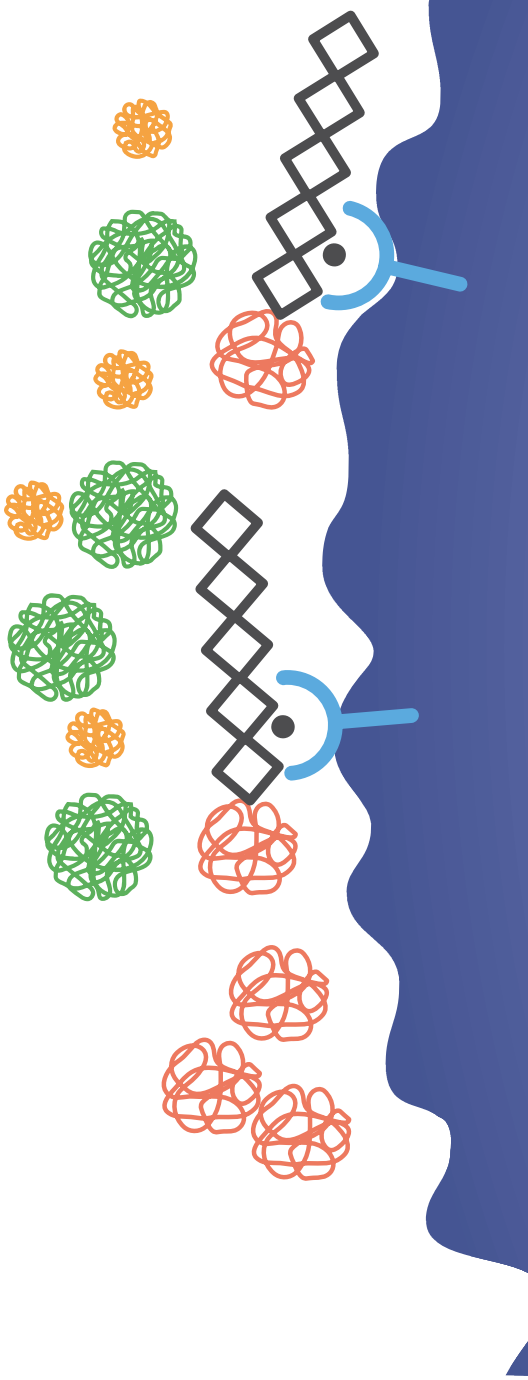
one step purification of His-tagged proteins

▶ BioFox 40/1200 IDAhigh/low and TRENhigh/low

High throughput agarose-based media

Immobilized metal ion affinity chromatography is based on a high affinity binding of an immobilized metal ion by chelating a part of the target protein. Performed on a preparative chromatographic medium, IMAC is a highly efficient procedure to purify histidine-tagged proteins from a cell extract in just one step.
Typical metal ions such as nickel and cobalt selectively retain histidine-tagged proteins. Recombinant antibodies can also be purified by IMAC. In general, IMAC purification is the preferred technique when high yields of pure and active protein are required.





Most frequently used metal ions for the purification are Zn^{2+}, Ni^{2+}, Co^{2+}, Ca^{2+}, Cu^{2+}, and Fe^{3+}. Ni^{2+} and Co^{2+} ions are commonly used for histidine-tagged proteins whereas Fe^{2+} and Ca^{2+} ions are used for unknown binding characteristics of a target protein. Co^{2+} and Zn^{2+} ions strongly bind untagged proteins as well as histidine-tagged proteins.

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

BioFox 40 IMAC media for immobilized metal ion chromatography (IMAC) are manufactured 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 up to 40 bar (580 psi) for high throughput biochromatography.

Two chelators are available for preparative scale IMAC: BioFox 40/1200 IDA and BioFox 40/1200 TREN.

Because sometimes the ligand density can have a great impact on the separation, BioFox 40/1200 IDA and BioFox 40/1200 TREN media are available with low and high metal ion loading capacities to allow for maximum flexibility when selecting the optimum IMAC conditions.

BioFox 40/1200 IDA

- Made from agarose, well-established and well-known in the biotechnology industry
- Choice of IMAC chemistry to selectively bind a large range of proteins
- High flow characteristics
- Strong binding

BioFox 40/1200 TREN

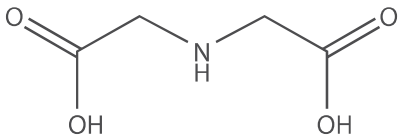
- Made from agarose, well-established and well-known in the biotechnology industry
- Choice of IMAC chemistry to selectively bind a large range of proteins
- High flow characteristics
- Weak binding

Therefore, the chelating efficiency is improved due to better access of proteins to the chelating sites.

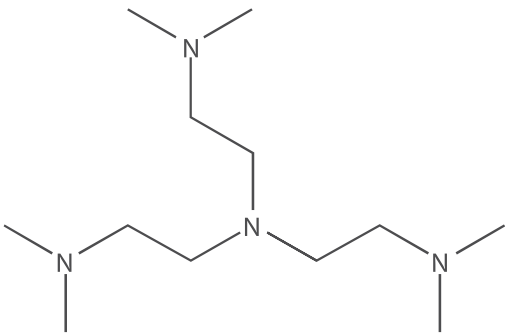
Because TREN shows a weaker binding, the non-specific binding of other proteins with intrinsic histidine groups together with the target protein is reduced.

Chelating groups

Iminodiacetic acid (IDA)



Tris(2-ethylaminoethyl) amine (TREN)



For further reading, we recommended the following article: "How to use immobilized metal ion affinity chromatography", A companion to Methods in Enzymology 4, 4-134 (1992) by Joy J. Winzerling et al.

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Tips for accurate IMAC operation

Selected metal ions

Most frequently used metal ions for the purification with IMAC are Zn^{2+}, Ni^{2+}, Co^{2+}, Ca^{2+}, Cu^{2+}, and Fe^{2+} , but principally all metal ions known to interact with proteins can be used.
For BioFox 40/1200 IMAC media,the metal ion capacity is only specified for copper and will vary slightly for other metal ions.

Metal ion loading

A 50 mM solution of a selected metal ion is prepared in distilled water. Some care has to be taken when selecting the loading buffer. The metal ion concentration will be rather high when adsorbed to the gel and precipitation may occur. Normally, a 0.1 M sodium acetate buffer at pH 5.5 can be used. The metal salt solution is loaded via a sample loop by repeated injections into the column until the media is fully loaded.

Removal of metal ions

Many metal ions undergo redox reactions and this may cause deviations during storage of the media. If the gel is not going to be used for a long period, it is recommended to remove the metal ions from the column bed. This is easily done with 0.1 M of ethylendiaminetetraacetic acid (EDTA) solution, either through repeated injections via sample loop or by directly pumping the eluent through the column.

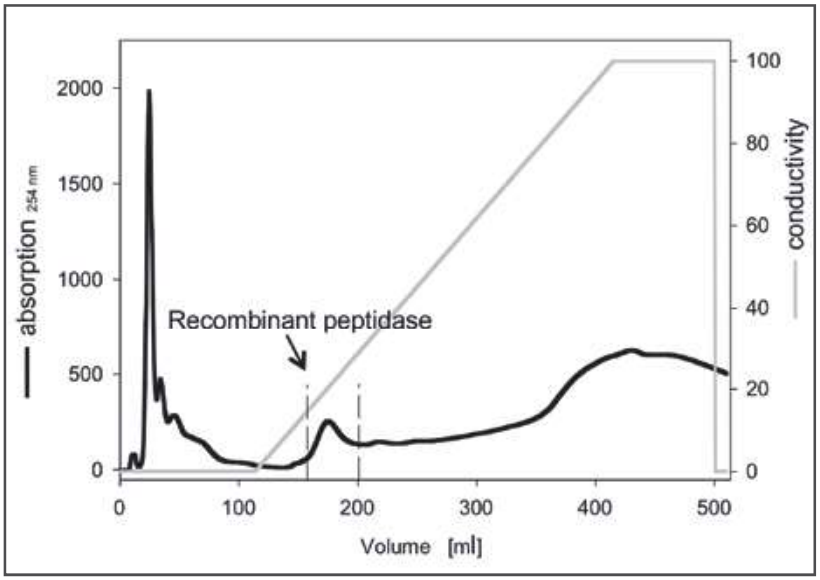
According to the separation problem, operating conditions may vary.
For adsorption, normally aqueous solvents could be applied, but organic solvents in low concentrations can also be used. Depending on the chelator's nature, both electrostatic and hydrophobic ineractions may be involved in the chelating complex formation. Additional care must also be taken regarding the ionic strength of the buffer solution. Buffers containing competitive groups with affinity for metal ions, such as imidazole, should be avoided.

Competitive elution with ammonium salts or imidazole buffer as well as decreasing pH value will elute the bound protein.

Applications

Because not all proteins show the same behaviour, four different IMAC chemistries are available. It is recommended to start with IDAhigh as this medium has the highest capacity and works well for most of the proteins. If the proteins are difficult to desorb from the column or elute with less activity, this reveals too strong binding. In this case, please try IDAlow. If this medium also fails, then try TRENhigh followed by TRENlow. The sequence will allow you to determine for the best separation medium for your application.

The Bioline Rack – with glass columns



Purification of a recombinant peptidase using preparative BioFox 40/1200 TRENhigh

Cleaning a recombinant peptidase (His-Tag®) from *E.coli* using BioFox 40/1200 TRENhigh (loaded with $NiSO_4$)

Media characteristics	BioFox 40 IDA	BioFox 40 TREN
Chelating group	Iminodiacetic acid (IDA)	Tris(2-ethylaminoethyl)amine (TREN)
Metal ion capacity µeqv	10–20 Cu^{2+}/ml IDAlow	10–20 Cu^{2+}/ml TRENlow
	40–50 Cu^{2+}/ml IDAhigh	50–60 Cu^{2+}/ml TRENhigh
Max flow rate at 20 cm bed height	> 500 cm/h	
Particle size	32–60 µm	
Agarose content	7.4–7.8 %	
pH stability	2–14	
Solvent stability after coupling the ligand	100 % methanol, 100 % ethanol, 8 M urea, 6 M guanidine hydrochloride, 30 % acetonitrile, 70 % formic acid, 30 % trifluoroacetic acid	

Media characteristics	BioFox 40/1200 Ni
pre-loaded metal ion	Nickel
Protein capacity	< 60 mg/ml
Max flow rate at 20 cm bed height	> 500 cm/h
Particle size	32–60 µm
Agarose content	7.4–7.8 %
pH stability	2–14
Solvent stability	100 % methanol, 100 % ethanol, 8 M urea, 6 M guanidine hydrochloride, 30 % acetonitrile, 70 % formic acid, 30 % trifluoroacetic acid

BioFox 40/1200 IDA and BioFox 40/1200 TREN media are supplied in aqueous suspensions with 22 % ethanol as preservative and are immediately ready for use after washing.

Ordering information BioFox 40/1200 IDAhigh/low	
Order No.	**Volume**
00IX39DBFZ	BioFox IDAhigh, 25 ml
00LX39DBFZ	BioFox IDAhigh, 150 ml
00QX39DBFZ	BioFox IDAhigh, 1 l
00IX39EBFZ	BioFox IDAlow, 25 ml
00LX39EBFZ	BioFox IDAlow, 150 ml
00QX39EBFZ	BioFox IDAlow, 1 l

Ordering information BioFox 40/1200 TRENhigh/low	
Order No.	**Volume**
00IX39BBFZ	BioFox 40/1200 TRENhigh, 25 ml
00LX39BBFZ	BioFox 40/1200 TRENhigh, 150 ml
00QX39BBFZ	BioFox 40/1200 TRENhigh, 1 l
00IX39CBFZ	BioFox 40/1200 TRENlow, 25 ml
00LX39CBFZ	BioFox 40/1200 TRENlow, 150 ml
00QX39CBFZ	BioFox 40/1200 TRENlow, 1 l

Ordering information BioFox 40/1200 Ni	
Order No.	**Volume**
00IX39FBFZ	BioFox 40/1200 Ni, 25 ml
00LX39FBFZ	BioFox 40/1200 Ni, 150 ml
00QX39FBFZ	BioFox 40/1200 Ni, 1 l

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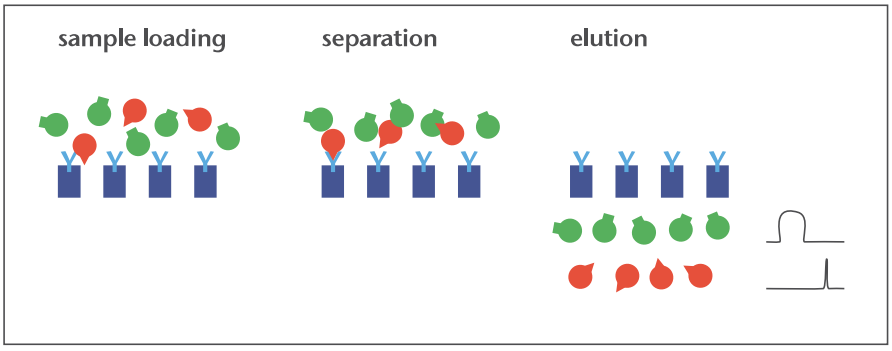
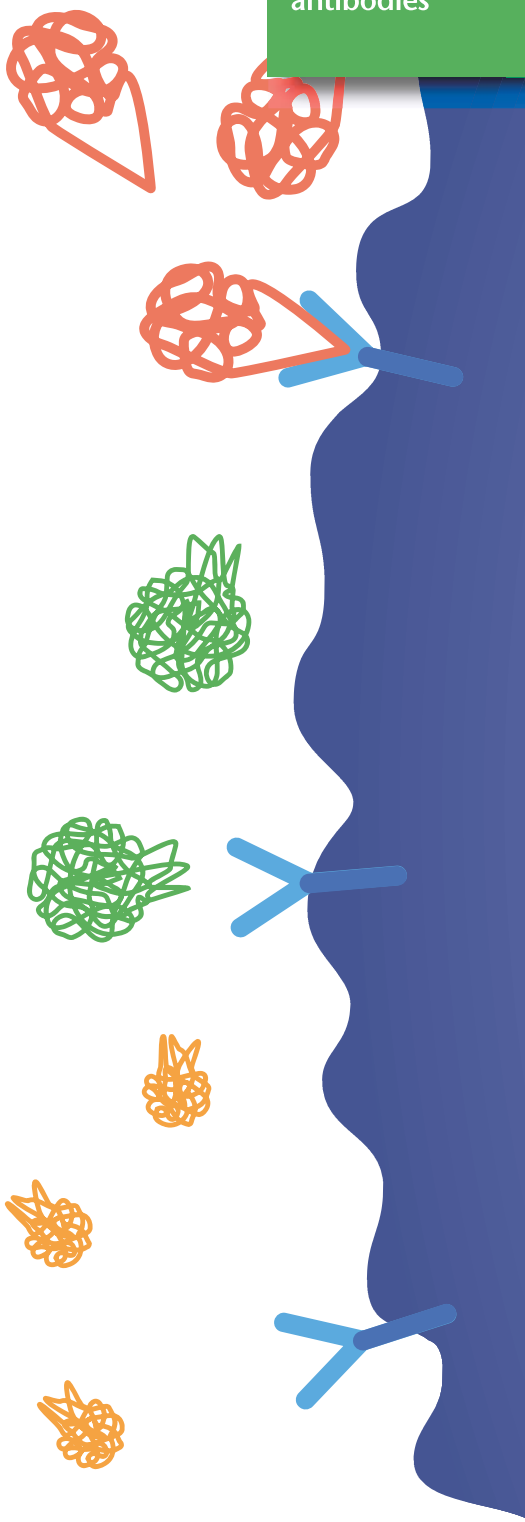
BioFox 40/1200 ACT and BioFox 40/10000 ACT

Analytical and preparative separations of proteins

Pre-activated media for preparative and bioprocess scale affinity chromatography with user's choice of ligands





Affinity chromatography separates proteins on the basis of a reversible interaction between a protein and a specific ligand covalently bound to a chromatographic medium. Thus, the typical affinity chromatography is often referred to as "lock and key principle" of a part of a molecule (key) with an immobilized ligand (lock). Antibodies, antigens, enzymes, short nucleic acids or peptides can be used as affinity ligands. They are coupled via their reactive functional groups such as amino, carboxyl, hydroxyl of thiol moieties to the gel. In particular, antigens or antibodies as ligands create a highly selective media for immunoaffinity purification. After loading the protein mixture into the separation column, non-binding molecules are almost entirely eliminated using rinse buffers, so that the final elution fraction contains the target molecule in high purity.

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

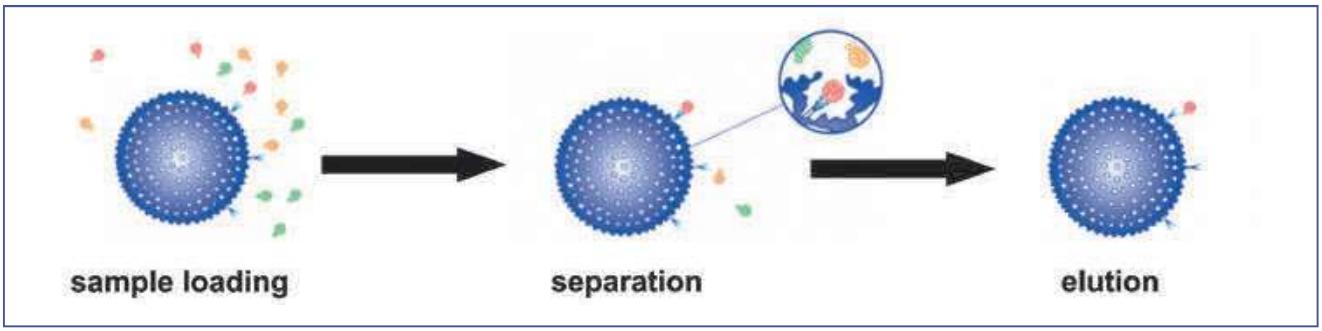
BioFox 40 ACT pre-activated separation media is produced from agarose using a proprietary cross-linking method that results in a highly porous and physically stable matrix. Besides the well-known selectivity of agarose, BioFox 40 ACT is pressure resistant up to 40 bar (580 psi) for high throughput biochromatography.

BioFox 40 ACT is activated according to the bromhydrin method. This activation method is, although proprietary, based upon well-understood chemistry, allowing you to perform the coupling reaction in an aqueous solution.

Step 1: Preparation of an immunoadsorbent with BioFox 40 ACT

Matrix-$OCH_2CH(OH)CH_2Br$ + Nu^- (e.g. –SH, $–NH_2$ or –OH) ——> Matrix-$OCH_2CH(OH)CH_2$-Nu
(Nu = Nucleophiles)

Step 2: Coupling reaction in aqueous solution



Since no toxic chemicals are necessary for the coupling procedure and the BioFox ACT products are stable at room temperature, coupling can easily be performed on your bench and at room temperature.

BioFox 40 ACT

- Made from agarose, well-established and well-known in the biotechnology industry
- Simple coupling procedures at room temperature
- Stable at room temperature in aqueous solution and at neutral pH
- Suitable for coupling of ligands containing sulfhydryl, amino or hydroxyl groups
- Optimum particle size of 40 µm and narrow particle size distribution for high throughput preparative and bioprocess scale applications

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Coupling conditions and selection of coupling buffers

BioFox 40 ACT comes ready to use. Proteins or other biomolecules with free amino and thiol groups will easily couple to BioFox 40 ACT. Just add the ligand to the suspension, stir and incubate overnight.

Hydroxyl groups can also be used for coupling, but will require pH 12 which is not compatible with most proteins. However, stable molecules can be coupled using the hydroxyl group. Remaining reactive groups are deactivated using 2-mercaptoethanol or ethanolamine.

Stability

BioFox 40 ACT media is stable for 12 months in aqueous solutions containing 22% ethanol at neutral pH and at room temperature without any significant decrease of coupling activity. The choice of storage buffer for a coupled gel medium depends on the properties of the ligand.

Type of ligand	Functional group of ligand	Coupling buffers
Organic molecules, peptides	Thiol-SH	pH 7 and higher. Sensitive ligands can be coupled at pH 7 but a better yield is achievable at higher pH. Basicity of the ligand will determine the coupling pH.
Organic molecules, peptides	Amines: primary ($-NH_2$) secondary (-NHR) tertiary ($-NR_3$)	When the ligand is used in excess, dissolve the ligand in distilled water and the basicity of the ligand will determine the coupling pH.
Proteins, polypeptides	Thiol (-SH)	pH 7 and higher. Sensitive ligands can be coupled at pH 7 but a better yield is achievable at higher pH.
Proteins, polypeptides	Primary amines ($-NH_2$)	Coupling yield will increase at higher pH. A carbonate buffer of pH 8 to 8.5 often gives sufficient coupling without denaturation of sensitive polypeptides and proteins. Another possibility is to run the coupling reaction at lower temperature.
All types	Hydroxyl (-OH)	The low nucleophilicity of the hydroxyl group requires coupling conditions at very high pH > 12. At a pH < 12 cross-linking and hydrolysis will compete with the coupling procedure.

The Bioline Rack – with glass columns

Media characteristics	BioFox 40/1200 ACT
Exclusion limit	1 200 kDa
Max flow rate at 20 cm bed height	> 500 cm/h
Particle size	32–60 µm
Spacer arms	4–16 # Atoms
Agarose content	7.4–7.8 %
Coupling groups	-OH, -NH_2, -SH
Degree of substitution	0.6–0.7 mol/mol
Solvent stability	100 % methanol, 100 % ethanol, 8 M urea, 6 M guanidine hydrochloride, 30 % acetonitrile, 70 % formic acid, 30 % trifluoroacetic acid

Media characteristics	BioFox 40/10000 ACT
Exclusion limit	10 000 kDa
Particle size	32–60 µm
Spacer arms	4–16 # Atoms
Agarose content	4.6–5.0 %
Coupling groups	-OH, -NH_2, -SH
Degree of substitution	0.6–0.7 mol/mol
Solvent stability	100 % methanol, 100 % ethanol, 8 M urea, 6 M guanidine hydrochloride, 30 % acetonitrile, 70 % formic acid, 30 % trifluoroacetic acid

BioFox 40/1200 ACT and BioFox 40/10000 ACT media are supplied as an aqueous suspension with 22 % ethanol as a preservative. After washing the media are immediately ready for use.

Ordering information BioFox 40/1200 ACT	
Order No.	**Volume**
00JX40MBFZ	Bulk media, 50 ml
00OX40MBFZ	Bulk media, 300 ml
00QX40MBFZ	Bulk media, 1 l
00RX40MBFZ	Bulk media, 5 l

Ordering information BioFox 40/10000 ACT	
Order No.	**Volume**
00JX40NBFZ	Bulk media, 50 ml
00OX40NBFZ	Bulk media, 300 ml
00QX40NBFZ	Bulk media, 1 l
00RX40NBFZ	Bulk media, 5 l

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