

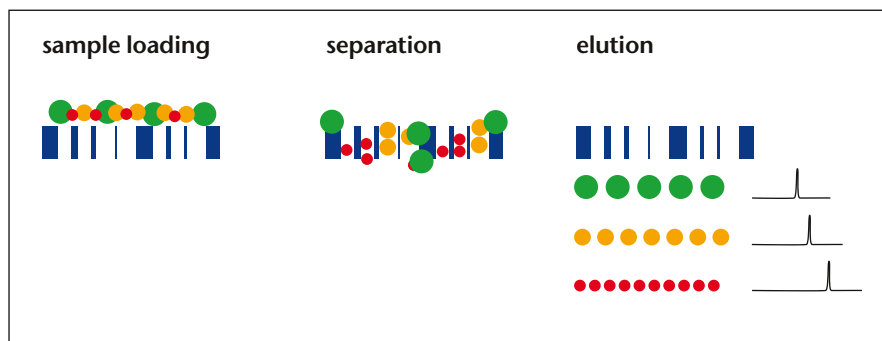
# Size exclusion chromatography (SEC)

## ► BioFox 17 SEC and 40 SEC

separation  
according to  
size

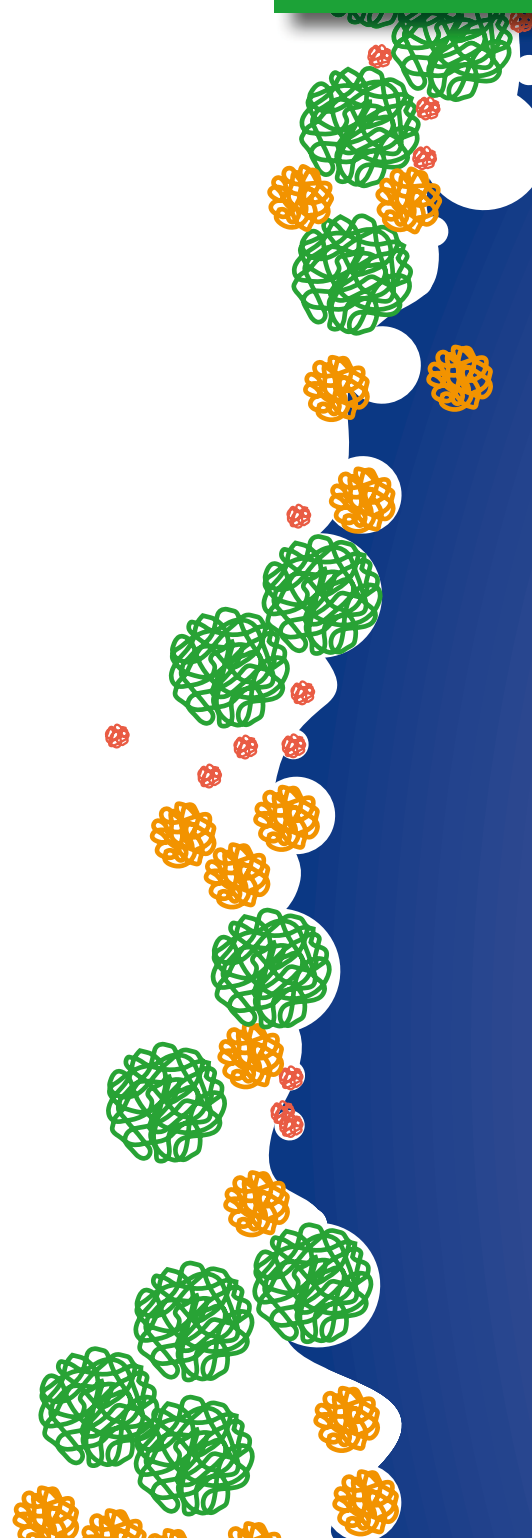
Analytical and preparative separations of proteins

Size exclusion chromatography separates molecules according to their different sizes. This technique is used in the first, intermediate or 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 occurring 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 enables non-denaturing mobile phases. Unlike media made from synthetic polymers, agarose does not have micro pores that can contribute to local pH variations in the micro-environment in the column thus leading to distorted separations.



# 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 and well-known 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)

Significantly save time and improve the performance of your bioseparations!

Packing technique	Standard procedure	KNAUER high-pressure packing
Filling pressure Filling duration <sup>1</sup>	Atmospheric pressure 12 h	15 bar (218 psi) 2 h
Separation performance		
Theoretical plates (ASTM) <sup>2</sup>	2138	5003

1) BioFox 40 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



The Bio  
with gla

### BioFox 17 SEC filtration gel

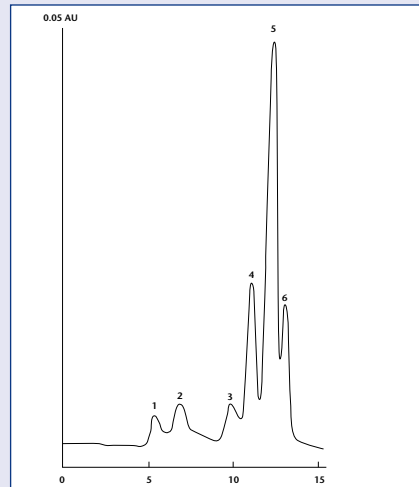
BioFox 17 SEC filtration gel has a higher selectivity for proteins, in comparison to matrices made from synthetic polymers. Consequently this SEC media has the capacity to successfully separate proteins, even when loading high amounts of protein. The small particle size of 17  $\mu\text{m}$  and the 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). Therefore BioFox 17 SEC was developed for high performance protein separations under varying conditions. Due to the high resolution, sharp and well-separated peaks are achieved, which makes the media ideal for analytical and semi-preparative purposes.

### BioFox 40 SEC filtration gel

BioFox 40 SEC filtration gel for preparative scale separations has an optimum particle size distribution of around 40  $\mu\text{m}$ . In combination with the proprietary cross-linking which increases the pressure stability, this media is easily packed in columns with very high efficiency and good flow characteristics. The high resolution that can be achieved makes it ideal for both lab scale preparative work and process scale separation of proteins.

### Separation of molecular weight standards on BioFox 17 SEC



#### Separation column

BioFox 17 SEC, 8 x 300 mm  
pre-packed column, 15 ml

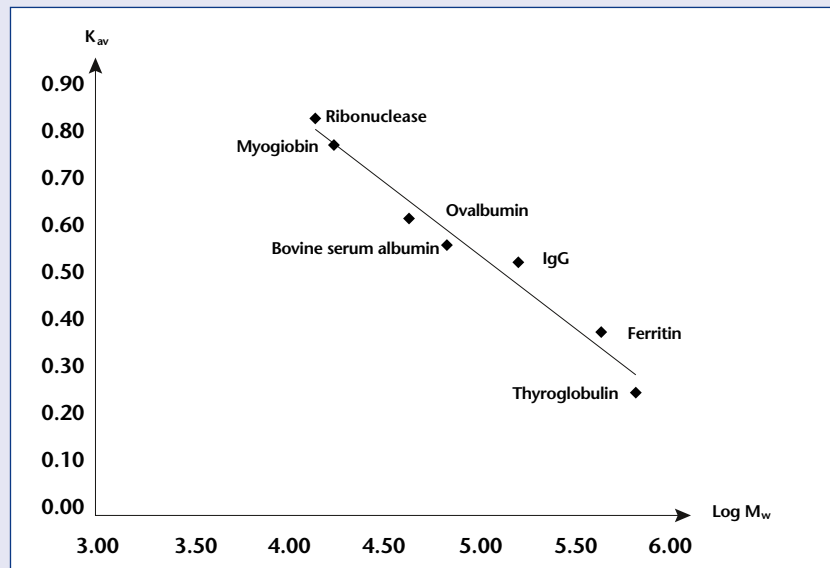
#### Separation conditions

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 17 and 40 SEC



$K_{av}$  (partition coefficient) is plotted against the logarithm of molecular weight for selected proteins. The selectivity curve is straight over the range  $K_{av}=0.2$  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.

## HPLC · SMB · Osmometry

Media characteristics	BioFox 17 SEC	BioFox 17/100	BioFox 40 SEC	BioFox 40/100	BioFox 40/10000
Exclusion limit [kDa]	1200	150	1200	150	10000
Max flow rate in an 8x300 mm column [cm/min]	–		15		10
Particle size [µm]	16 – 18		32 – 60		
Agarose content [%]	7.4–7.8	9.2–9.5	7.4–7.8	9.2–9.8	4.6-5.0
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 SEC	BioFox 17/100	BioFox 40 SEC	BioFox 40/100	BioFox 40/10000
Plates/m	25 000 – 30 000		8 000 – 11 000		
Mesh size of the net [µm]	10				
Optimal operating flow rate [ml/min]	0.5–2.0				
Maximum operating flow rate [ml/min]	6				
pH stability	1 – 14				
Asymmetry	0.85 – 1.15				
Operating temperature [°C]	4–40				
Cleaning	Columns can be sanitized with 1 M NaOH or 70% ethanol.				
Materials in contact with eluent	Borosilicate glass (chromatographie tube), titanium (filter), PEEK (polyetheretherketone) (tubing), EPDM (O-ring), PVDF (polyvinylidifluoride) (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 SEC	BioFox 17/100	BioFox 40 SEC
	Size exclusion agarose media – pre-packed in analytical grade columns – preserved with 22% ethanol		
Media volume [ml]	15, 25		15
Internal diameter [mm]	8		8
Bed height [mm]	300, 500		300

Ordering information		Ordering information	
Order No.	Column size/volume	Order No.	Column size/volume
Y4007	Pre-packed column, 15 ml (8x300mm) (17 SEC)	Y4001	Bulk media, 1 l (40 SEC)
Y4010	Pre-packed column, 25 ml (8x500mm) (17 SEC)	Y4043	Bulk media, 5 l (40 SEC)
Y4017	Pre-packed column, 15 ml (8x300 mm) (17/100 SEC)	Y4044	Bulk media, 25 ml (40/100 SEC)
Y4040	Pre-packed column, 15 ml (8x300mm) (40 SEC)	Y4045	Bulk media, 300 ml (40/100 SEC)
Y4041	Bulk media, 25 ml (40 SEC)	Y4046	Bulk media, 1 l (40/100 SEC)
Y4042	Bulk media, 300 ml (40 SEC)	Y4049	Bulk media, 1 l (40/10000 SEC)

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

Visit [www.knauer.net](http://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](mailto:info@knauer.net)  
Internet: [www.knauer.net](http://www.knauer.net)

