

# Size exclusion chromatography

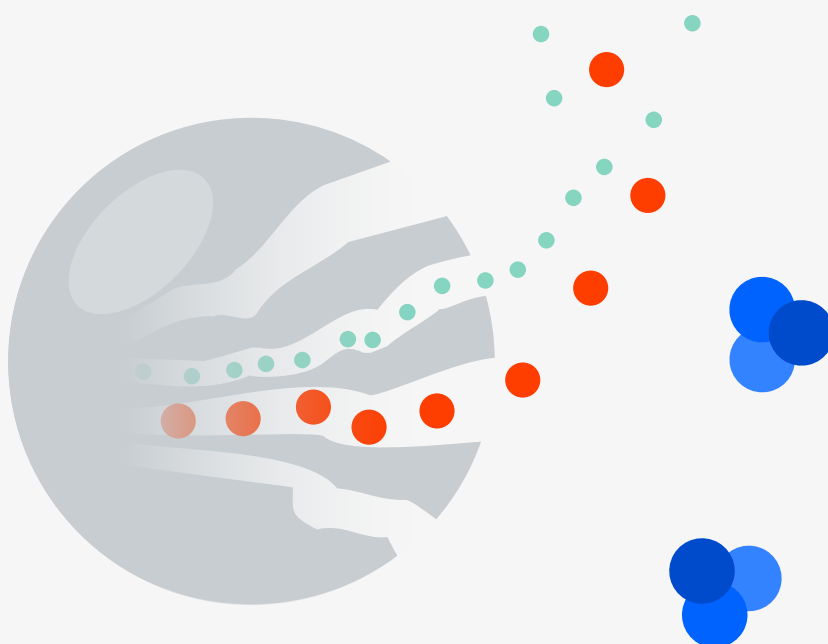
Size exclusion chromatography (SEC) (also called gel filtration, GF) separates molecules on the basis of differences in size. As this is a non-binding technique, the choice of running buffers is large and can be optimized for the target molecule. For best resolution when using SEC, a slow flow rate and a sample volume of maximum 4% of the column volume should be used. This technique is therefore best suited for the polishing step in a purification process.

## Target molecules

### Polishing

Proteins, peptides, viruses, tagged proteins and oligonucleotides. Several different pore sizes are available of WorkBeads SEC resins which makes them suitable for a large range of target molecules of different sizes. WorkBeads 200 SEC is designed with a larger bead size optimized for separation of viscous samples,

See schematic depicting size exclusion chromatography.



**WorkBeads 40/100 SEC**  
**WorkBeads 40/1000 SEC**  
**WorkBeads 40/10 000 SEC**  
**WorkBeads Macro SEC**  
**WorkBeads 200 SEC**

- Produced using a proprietary cross-linking method that results in highly porous and physically stable matrices
- Availability in several different porosities give robust and wide separation ranges
- Alternative bead sizes for viscous samples
- Resistant to harsh cleaning agents (NaOH)
- Available in several different GoBio prepacked columns

## Comparison of WorkBeads SEC resins

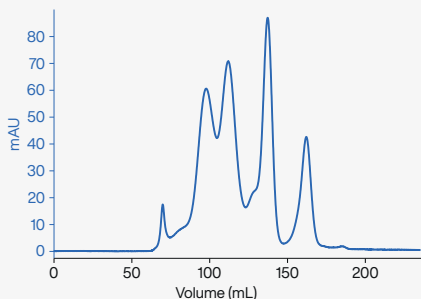
	Average bead size, $\mu\text{m}$	Separation range, kD	Exclusion limit, kD	Separation range, D				
				$10^4$	$10^5$	$10^6$	$10^7$	$10^8$
WorkBeads 40/100 SEC	45	10 – 150	150					
WorkBeads 40/1000 SEC	45	10 – 1200	1200					
WorkBeads 40/10 000 SEC	45	10 – 10 000	10 000					
WorkBeads Macro SEC	45	10 – 30 000	30 000					
WorkBeads 200 SEC	180	10 – 6000	6000					

## Applications

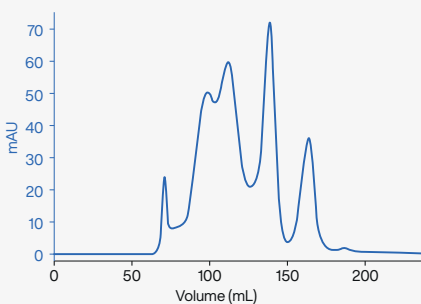
### WorkBeads 40/1000 SEC, different flow rates

Resin: WorkBeads 40/1000 SEC  
 Column: 16 x 950 mm, 181 mL  
 Sample: 250  $\mu\text{L}$ , 0.5 mg/mL thyroglobulin, 0.5 mg/mL ferritin, 0.5 mg/mL ovalbumin and 0.5 mg/mL ribonuclease A (in order of elution)  
 Buffer: PBS, pH 7.2  
 Flow rates: 25 cm/h (0.84 mL/min)  
 50 cm/h (1.68 mL/min)  
 100 cm/h (3.35 mL/min)

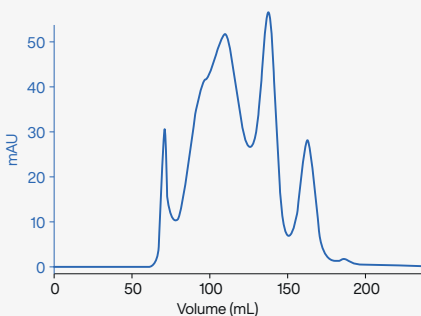
#### 25 cm/h



#### 50 cm/h



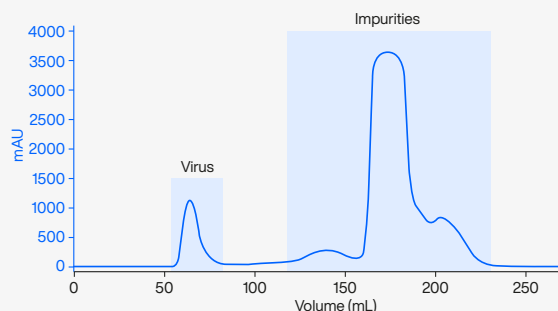
#### 100 cm/h



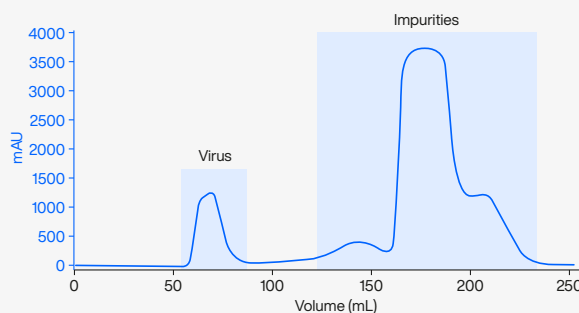
### Purification of animal viral vaccine

Resin: WorkBeads 40/1000 SEC  
 Sample: Inactivated rabies virus (A) 10 mL (5.7% of CV) (B) 15 mL (8.5% of CV)  
 Column: 16 x 880 mm, 176 mL  
 Flow rate: 5 mL/min, 150 cm/h  
 Buffer: PBS, pH 7.2

#### (A) Sample volume 5.7% of CV



#### (B) Sample volume 8.5% of CV



# Technical specifications

	WorkBeads 40/100 SEC	WorkBeads 40/1000 SEC	WorkBeads 40/10 000 SEC
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Separation range <sup>1</sup>	10 to 150 kD	10 to 1200 kD	10 to 10 000 kD
Exclusion limit	150 kD	1200 kD	10 000 kD
Average particle size <sup>2</sup> ( $D_{v50}$ )	45 $\mu$ m	45 $\mu$ m	45 $\mu$ m
Recommended flow rate <sup>3</sup>	15 to 150 cm/h	15 to 150 cm/h	15 to 150 cm/h
Maximum flow rate <sup>4,5</sup>	600 cm/h	600 cm/h	300 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Should not be stored at low pH for prolonged time.		
pH stability	2 to 13	2 to 13	2 to 13
Storage	2 to 25°C in 20% ethanol	2 to 25°C in 20% ethanol	2 to 25°C in 20% ethanol

<sup>1</sup> Globular proteins.

<sup>2</sup> The median particle size of the cumulative volume distribution.

<sup>3</sup> The flow rate is important for the resolution and a lower flow rate often gives an increased resolution. A higher flow rate can be used during equilibration to speed up the separation.

<sup>4</sup> Determined in water using a 25 × 200 mm column.

<sup>5</sup> Note: Make sure that the column hardware max pressure is not exceeded.

	WorkBeads Macro SEC	WorkBeads 200 SEC
Separation range <sup>1</sup>	10 to 30 000 kD	10 to 6000 kD
Exclusion limit	30 000 kD	6000 kD
Matrix	Highly cross-linked agarose	Highly cross-linked agarose
Average particle size <sup>2</sup> ( $D_{v50}$ )	45 $\mu$ m	180 $\mu$ m
Recommended flow rate <sup>3</sup>	15 to 150 cm/h	15 to 150 cm/h
Max flow rate <sup>4,5</sup>	300 cm/h	900 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Should not be stored at low pH for prolonged time.	
pH stability	2 to 13	2 to 13
Storage	2 to 25°C in 20% ethanol	2 to 25°C in 20% ethanol

<sup>1</sup> Globular proteins.

<sup>2</sup> The median particle size of the cumulative volume distribution.

<sup>3</sup> The flow rate is important for the resolution and a lower flow rate often gives an increased resolution. A higher flow rate can be used during equilibration to speed up the separation.

<sup>4</sup> Determined in water using a 25 × 200 mm column.

<sup>5</sup> Note: Make sure that the column hardware max pressure is not exceeded.

## Ordering information

Product name	Pack size	Article number
WorkBeads 40/100 SEC	25 mL	40 340 001
	300 mL	40 340 003
	1 L	40 340 010
	5 L	40 340 050
	10 L	40 340 060
WorkBeads 40/1000 SEC	25 mL	40 300 001
	300 mL	40 300 003
	1 L	40 300 010
	5 L	40 300 050
	10 L	40 300 060
WorkBeads 40/10 000 SEC	25 mL	40 350 001
	300 mL	40 350 003
	1 L	40 350 010
	5 L	40 350 050
	10 L	40 350 060
WorkBeads Macro SEC	25 mL	40 370 001
	300 mL	40 370 003
	1 L	40 370 010
	5 L	40 370 050
	10 L	40 370 060
WorkBeads 200 SEC	300 mL	20 300 003
	1 L	20 300 010
	5 L	20 300 050
	10 L	20 300 060

## More information

### Data Sheet, DS 40 300 010

WorkBeads 40/100 SEC, WorkBeads 40/1000 SEC,  
WorkBeads 40/10 000 SEC, WorkBeads Macro SEC,  
WorkBeads 200 SEC, GoBio prepacked columns

→ [bio-works.com/product/sec-resin](https://bio-works.com/product/sec-resin)

