Gel Filtration Calibration Kit LMW
Gel Filtration Calibration Kit HMW

SIZE EXCLUSION CHROMATOGRAPHY

Gel Filtration Calibration Kit LMW and HMW are available for the calibration of gel filtration (size exclusion chromatography, SEC) columns. The Low Molecular Weight (LMW) Kit (Fig 1) contains five proteins with M_r in the range 6500 to 75 000 and Blue Dextran 2000 (Table 1). The High Molecular Weight (HMW) Kit contains five proteins with M_r in the range 43 000 to 669 000 and Blue Dextran 2000 (Table 2).

The proteins used in the kits are suitable for calibration of columns packed with high-resolution SEC resin such as Superdex™ 75 Increase, Superdex 200 Increase, Superdex 75 prep grade, Superdex 200 prep grade, Superose™ 6 prep grade, Superose 6 Increase, Sephacryl™ S-100, Sephacryl S-200, and Sephacryl S-300 to allow accurate molecular weight determinations of proteins.

Gel Filtration Calibration Kit LMW and HMW offer:

- Well-defined protein standards that show excellent behavior in SEC and enable simple, reliable calibration of columns
- An optimized range of proteins that suits high-resolution resins and prepacked columns with molecular weight ranges from 6500 to 669 000
- Each kit contains five proteins that are lyophilized in individual vials
- Blue Dextran 2000 to determine the void volume in the column

Fig 1. Gel Filtration Calibration Kit LMW.

Table 1. Characteristics of Gel Filtration Calibration Kit LMW

Protein (weight per vial)	Molecular weight (M_r)	Source
Aprotinin (10 mg)	6500	Bovine lung
Ribonuclease A (50 mg)	13 700	Bovine pancreas
Carbonic anhydrase (15 mg)	29 000	Bovine erythrocytes
Ovalbumin (50 mg)	43 000	Hen egg
Conalbumin (50 mg)	75 000	Chicken egg white
Blue dextran 2000 (50 mg)	2 000 000	

Table 2. Characteristics of Gel Filtration Calibration Kit HMW

Protein (weight per vial)	Molecular weight (M_r)	Source
Ovalbumin (50 mg)	43 000	Hen egg
Conalbumin (50 mg)	75 000	Chicken egg white
Aldolase* (50 mg)	158 000	Rabbit muscle
Ferritin* (15 mg)	440 000	Horse spleen
Thyroglobulin (50 mg)	669 000	Bovine thyroid
Blue dextran 2000 (50 mg)	2 000 000	

* These proteins are supplied mixed with sucrose or mannitol to maintain stability and aid their solubility.

High-resolution determination of molecular weight

The use of SEC for the determination of the molecular weight and size of proteins is well documented. The technique is based on the established ability of high-resolution resins, such as Superdex, Superose, and Sephacryl to separate molecules according to size. Prepacked columns are available and can be run on chromatography systems such as, ÄKTA™ protein purification systems.

Molecular weight determination by SEC is carried out by comparing an elution volume parameter, such as the distribution coefficient (K_{av}) of the protein of interest, with the values obtained for several known calibration standards. In practice, a homologous series of globular proteins have a sigmoid relationship between their elution volume parameters and the logarithm of their molecular weights (M_r). The M_r of an unknown protein can be determined from the calibration curve (plot of K_{av} versus log M_r) once its K_{av} value is calculated from the measured elution volume. For accurate determination of M_r, the calibration standards must have the same relationship between molecular weight and molecular size as the substance of interest. Our calibration kits provide highly purified, well-characterized, globular protein standards for calibration of SEC columns.

Typical results

Typical calibration results from chromatographic runs and calculated calibration curves using prepacked Superdex Increase, Superose Increase, and Sephacryl columns are shown in Figures 2 to 11.

The method used for Figures 2 to 11:

Sample:	Proteins from Gel Filtration Calibration Kit LMW and HMW: aprotinin (Apr), RNase A (R), carbonic anhydrase (CA), ovalbumin (O), conalbumin (C), aldolase (Ald), ferritin (F), and thyroglobulin (T)	
Sample volume:	*Figures 2 to 4:*	100 µL
	Figure 5:	2.6 mL
	Figures 6 to 11:	500 µL
Buffer:	*Figures 1 to 4 and 6 to 11:*	50 mM phosphate buffer, 150 mM NaCl, pH 7.2
	Figure 5:	140 mM NaCl, 2.7 mM KCl, 10 mM sodium phosphate, pH 7.4
Flow rate:	*Figures 2, 4, 9, 10, and 11:*	0.5 mL/min
	Figure 3:	0.6 mL/min
	Figure 5:	2.7 mL/min
	Figure 6:	1.27 mL/min
	Figures 7 and 8:	1.0 mL/min
System:	ÄKTA pure 25	
Detection:	280 nm	

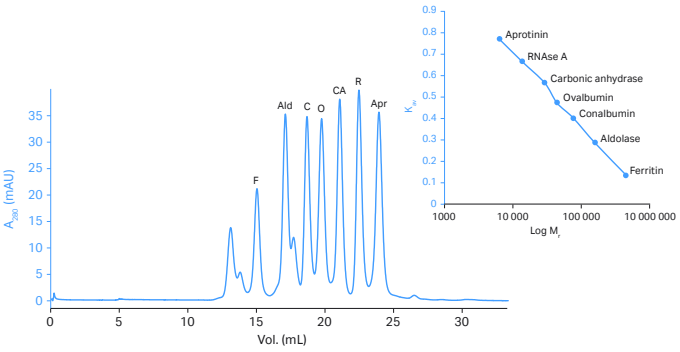

Fig 2. Chromatographic separation and calibration curve for the standard proteins on Superdex 200 Increase 10/300 GL column.

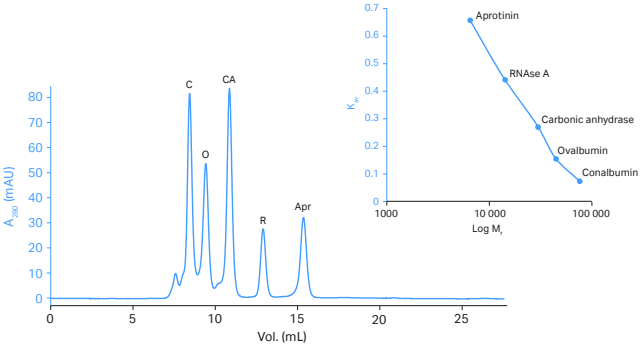

Fig 3. Chromatographic separation and calibration curve for the standard proteins on Superdex 75 Increase 10/300 GL column.

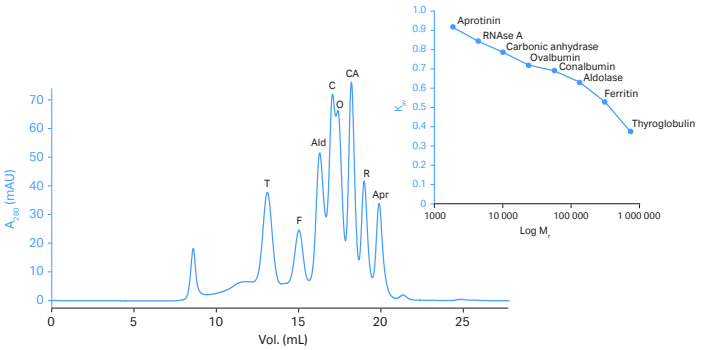

Fig 4. Chromatographic separation and calibration curve for the standard proteins on Superose 6 Increase 10/300 GL column. Note: thyroglobulin may be excluded from the calculation of K_{av} due to nonlinear behavior of thyroglobulin on this column. Thyroglobulin may however, be included in a plot of √-log (K_{av}) vs Stokes radius (R_{St}).

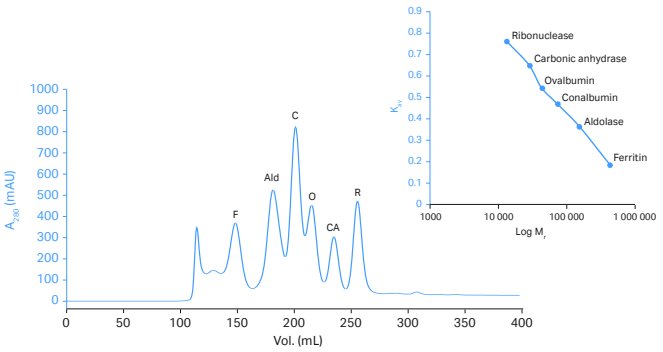

Fig 5. Chromatographic separation and calibration curve for the standard proteins on HiLoad™ 26/600 Superdex 200 pg column.

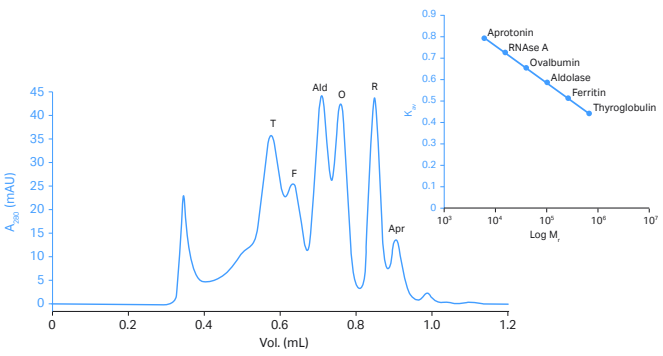

Fig 6. Chromatographic separation of the standard proteins on HiLoad 16/600 Superose 6 pg column.

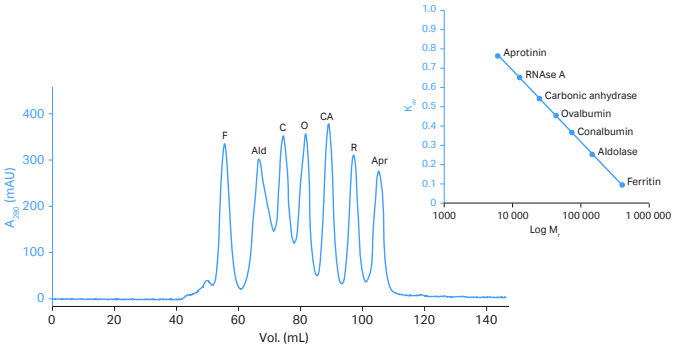

Fig 7. Chromatographic separation and calibration curve for the standard proteins on HiLoad 16/600 Superdex 200 pg column.

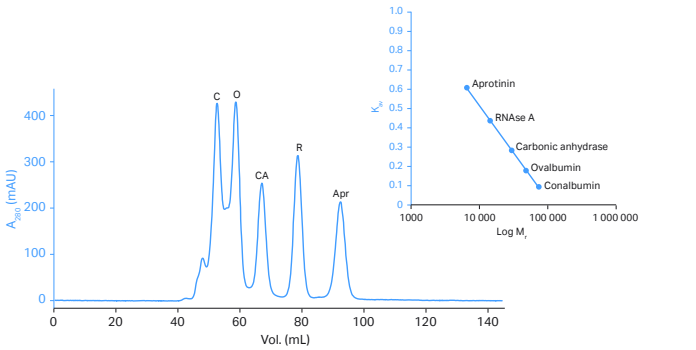

Fig 8. Chromatographic separation and calibration curve for the standard proteins on HiLoad 16/600 Superdex 75 pg column.

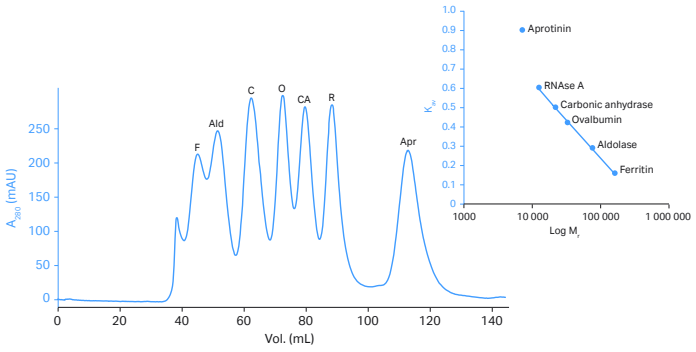

Fig 9. Chromatographic separation and calibration curve for the standard proteins on HiPrep™ 16/60 Sephacryl S-300 HR column. Note: aprotinin may be excluded from the calculation of K_{av} due to non-linear behavior of aprotinin on this column.

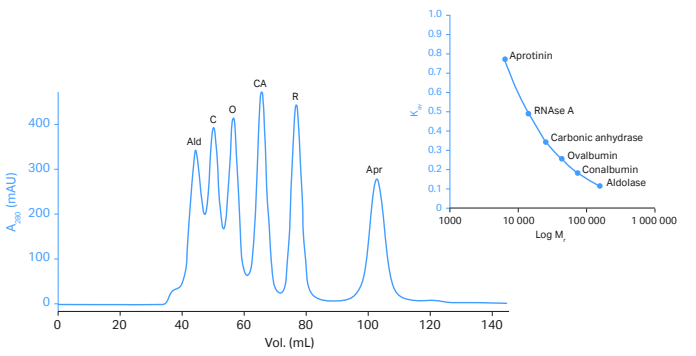

Fig 10. Chromatographic separation and calibration curve for the standard proteins on HiPrep 16/60 Sephacryl S-200 HR column.

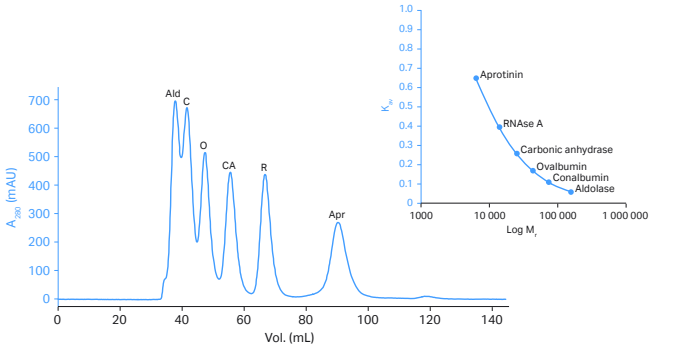

Fig 11. Chromatographic separation and calibration curve for the standard proteins on HiPrep 16/60 Sephacryl S-100 HR column.

Ordering information

Gel Filtration Calibration Kits	Quantity	Product code
Low Molecular Weight	1	28403841
High Molecular Weight	1	28403842

Related products		
Superdex 75 Increase 10/300 GL	1	29148721
Superdex 75 Increase 3.2/300	1	29148723
Superdex 200 Increase 10/300 GL	1	28990944
Superdex 200 Increase 3.2/300	1	28990946
HiLoad 16/600 Superose 6 pg	1	29323952
HiLoad 16/600 Superdex 75 pg	1	28989333
HiLoad 26/600 Superdex 75 pg	1	28989334
HiLoad 16/600 Superdex 200 pg	1	28989335
HiLoad 26/600 Superdex 200 pg	1	28989336
Superdex 30 Increase 10/300 GL	1	29219757
Superdex 30 Increase 3.2/300	1	29219758
Superose 6 Increase 10/300 GL	1	29091596
Superose 6 Increase 3.2/300	1	29091598
HiPrep 16/60 Sephacryl S-100 HR	1	17116501
HiPrep 26/60 Sephacryl S-100 HR	1	17119401
HiPrep 16/60 Sephacryl S-200 HR	1	17116601
HiPrep 26/60 Sephacryl S-200 HR	1	17119501
HiPrep 16/60 Sephacryl S-300 HR	1	17116701
HiPrep 26/60 Sephacryl S-300 HR	1	17119601

Related literature	
Selection guide: Size exclusion columns and resins	18112419
Handbook: Size Exclusion Chromatography, Principles and Methods	18102218

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