Gel Filtration Calibration Kit LMW
Gel Filtration Calibration Kit HMW

Two Gel Filtration Calibration Kits are available for the calibration of gel filtration columns. The Low Molecular Weight (LMW) Kit 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 gel filtration columns packed with high-resolution gel filtration media such as Superdex™ 75, Superdex 200, Superdex 75 prep grade, Superdex 200 prep grade, Superose™ 12, Superose 6, 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 gel filtration and enable simple, reliable calibration of gel filtration columns
- An optimized range of proteins that suits high-resolution media 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 fraction in the column

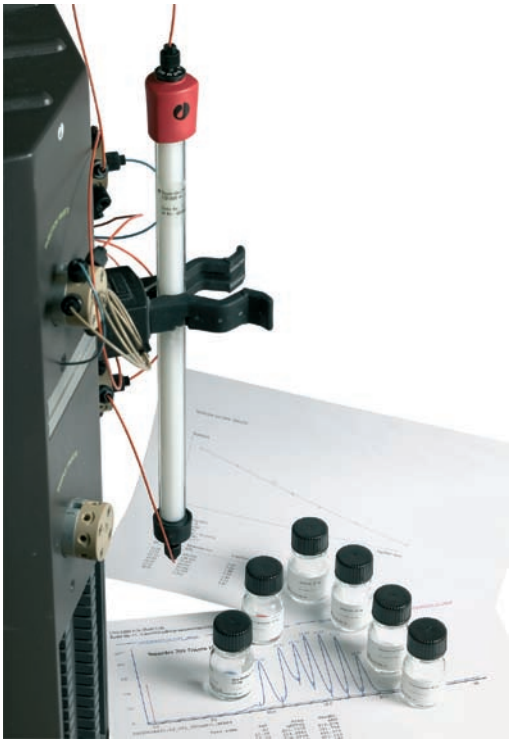

Fig 1. Gel Filtration Calibration Kit LMW and HMW are two kits for calibration of gel filtration columns.

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[1] (50 mg)	158 000	Rabbit muscle
Ferritin[1] (15 mg)	440 000	Horse spleen
Thyroglobulin (50 mg)	669 000	Bovine thyroid
Blue dextran 2000 (50 mg)	2 000 000	

[1] 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 gel filtration chromatography 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 gel filtration media, 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, ÄKTAdesign™.

Molecular weight determination by gel filtration is carried out by comparing an elution volume parameter, such as the gel phase 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. GE Healthcare Calibration Kits provide highly purified, well-characterized, globular protein standards for calibration of gel filtration columns.

Calibration with UNICORN software

Calibration of gel filtration columns on ÄKTAdesign systems can be supported by UNICORN™ software with the optional Analysis Module[1]. The Analysis Module automates molecular weight measurement of unknown proteins from calibration curves based on proteins of known M_r. The curves are prepared by plotting retention volume data against the logarithm of the molecular weights of the calibration kit proteins. Thereafter, molecular weights of unknown proteins can extrapolated from the curve (Fig 2) together with calculated correlation and variance.

[1] The Analysis Module must be ordered separately and installed after the regular UNICORN installation.

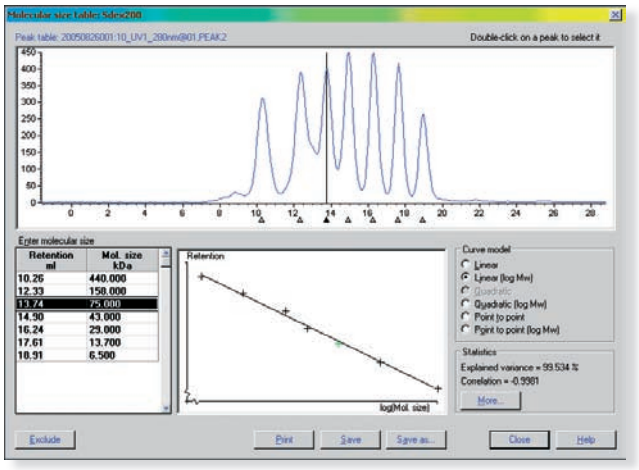

Fig 2. Molecular size table dialog box in the UNICORN software Analysis Module.

Typical results

Typical calibration results from chromatographic runs and calculated calibration curves using prepacked Superdex, Superose and Sephacryl columns are shown in Figures 3 to 11.

The method used for Figures 3 to 11:

Sample:	Proteins from Gel Filtration Calibration Kits LMW and HMW: aprotinin (Apr), RNase A (R), carbonic anhydrase (CA), ovalbumin (O), conalbumin (C), aldolase (Ald), ferritin (F) and thyroglobulin (T)	
Sample vol.:	*Figures 3 to 6:*	100 µl
	Figures 7 to 11:	500 µl
Buffer:	50 mM phosphate buffer, 150 mM NaCl, pH 7.2	
Flow rate:	*Figures 3, 5, 6, 9, 10 and 11:*	0.5 ml/min
	Figure 4:	0.6 ml/min
	Figures 7 and 8:	1.0 ml/min
System:	ÄKTAexplorer™ 10	
Detection:	280 nm	

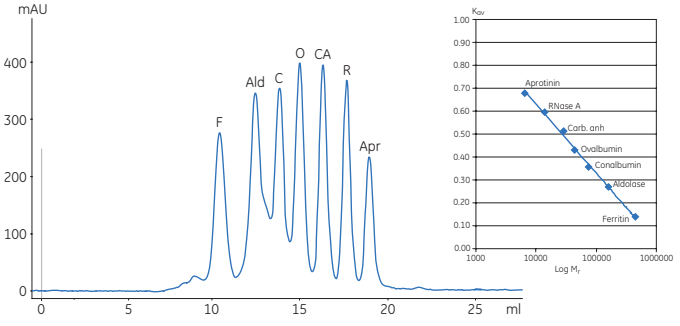

Fig 3. Chromatographic separation and calibration curve for the standard proteins on Tricorn™ Superdex 200 10/300 GL column.

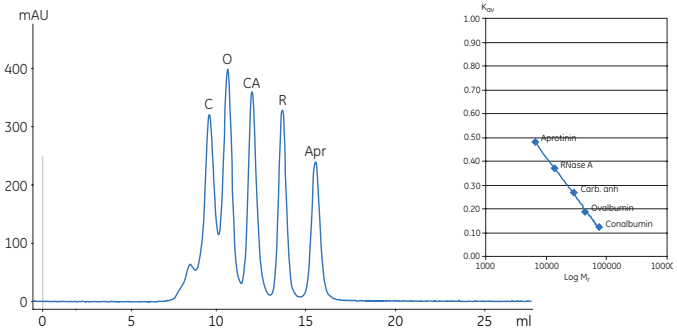

Fig 4. Chromatographic separation and calibration curve for the standard proteins on Tricorn Superdex 75 10/300 GL column.

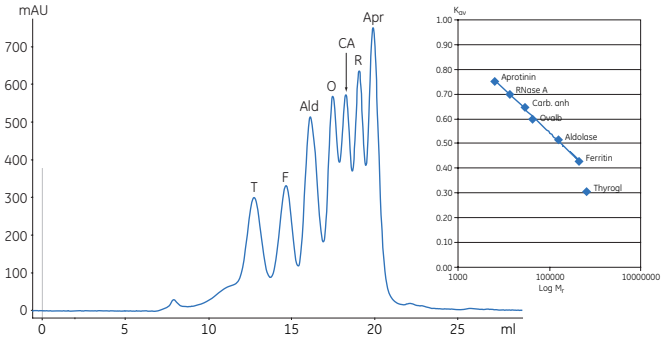

Fig 5. Chromatographic separation and calibration curve for the standard proteins on Tricorn Superose 6 10/300 GL column.

Note: Thyroglobulin may be excluded from the calculation of K_{av} due to non-linear behavior of thyroglobulin on this column. Thyroglobulin may however, be included in a plot of √-log (K_{av}) vs. Stokes Radius (R_{St}).

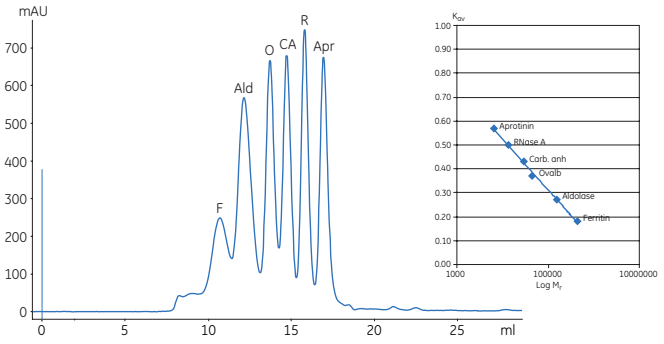

Fig 6. Chromatographic separation and calibration curve for the standard proteins on Tricorn Superose 12 10/300 GL column.

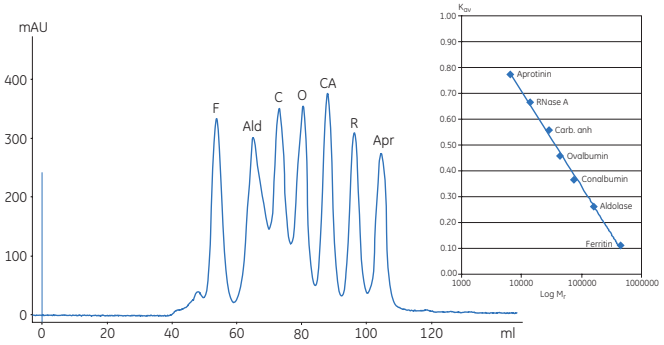

Fig 7. Chromatographic separation and calibration curve for the standard proteins on HiLoad™ 16/60 Superdex 200 pg column.

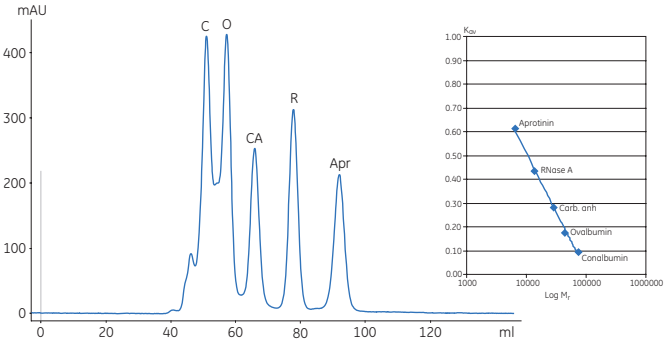

Fig 8. Chromatographic separation and calibration curve for the standard proteins on HiLoad 16/60 Superdex 75 pg column.

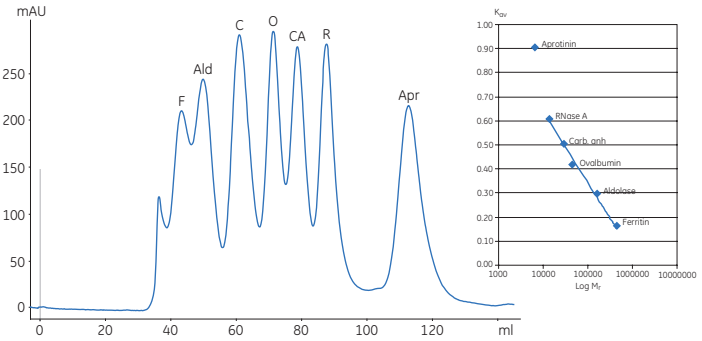

Fig 9. Chromatographic separation and calibration curve for the standard proteins on HiPrep™ 16/60 Sephacryl S-300 HR column.

Note: Aprotinin may be excluded from the calculation of K_{av} due to non-linear behavior of aprotinin on this column.

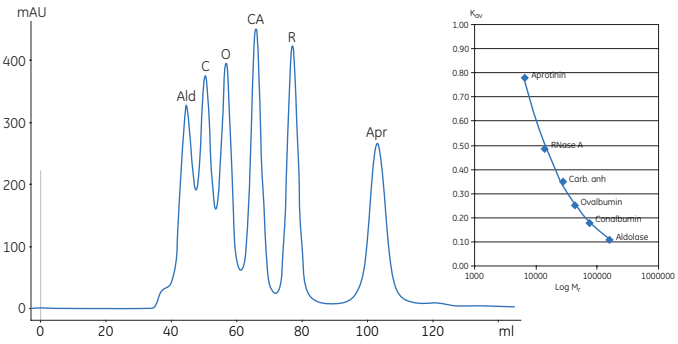

Fig 10. Chromatographic separation and calibration curve for the standard proteins on HiPrep 16/60 Sephacryl S-200 HR column.

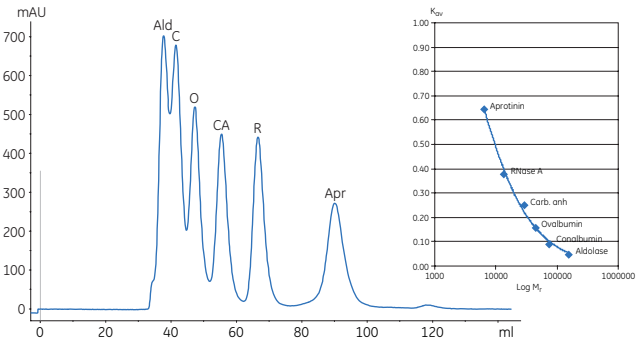

Fig 11. Chromatographic separation and calibration curve for the standard proteins on HiPrep 16/60 Sephacryl S-100 HR column.

Ordering information

Gel Filtration Calibration Kits	Quantity	Code no.
Low Molecular Weight	1	28-4038-41
High Molecular Weight	1	28-4038-42

Related literature	Code no.
Selection guide: Gel Filtration Columns and Media	18-1124-19
Handbook: Gel Filtration - Principles & Methods	18-1022-18

Related products

Quantity	Code no.	
Superdex 75 10/300 GL	1	17-5174-01
Superdex 75 PC 3.2/30	1	17-0771-01
Superdex 200 10/300 GL	1	17-5175-01
Superdex 200 PC 3.2/30	1	17-1089-01
HiLoad 16/60 Superdex 75 pg	1	17-1068-01
HiLoad 26/60 Superdex 75 pg	1	17-1070-01
HiLoad 16/60 Superdex 200 pg	1	17-1069-01
HiLoad 26/60 Superdex 200 pg	1	17-1071-01
Superose 12 10/300 GL	1	17-5173-01
Superose 12 PC 3.2/30	1	17-0674-01
Superose 6 10/300 GL	1	17-5172-01
Superose 6 PC 3.2/30	1	17-0673-01
HiPrep 16/60 Sephacryl S-100 HR	1	17-1165-01
HiPrep 26/60 Sephacryl S-100 HR	1	17-1194-01
HiPrep 16/60 Sephacryl S-200 HR	1	17-1166-01
HiPrep 26/60 Sephacryl S-200 HR	1	17-1195-01
HiPrep 16/60 Sephacryl S-300 HR	1	17-1167-01
HiPrep 26/60 Sephacryl S-300 HR	1	17-1196-01
UNICORN – Analysis Module	1	11-0003-60

www.gehealthcare.com/protein-purification
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28-4073-84 AA 06/2006

Elanders Österväla 2006