

# 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 <sub>r</sub> ) | 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<br>(weight per vial) | Molecular<br>weight (M <sub>r</sub> ) | 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.

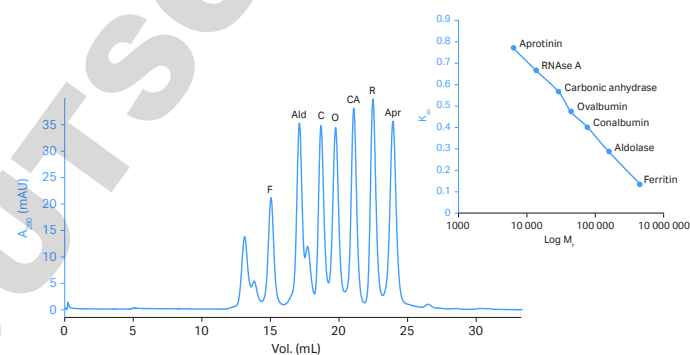
Molecular weight determination by SEC is carried out by comparing an elution volume parameter, such as the distribution coefficient ( $K_{av}$ ) of the protein of interest, with the values obtained for several known calibration standards. In practice, a homologous series of globular proteins have a sigmoid 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

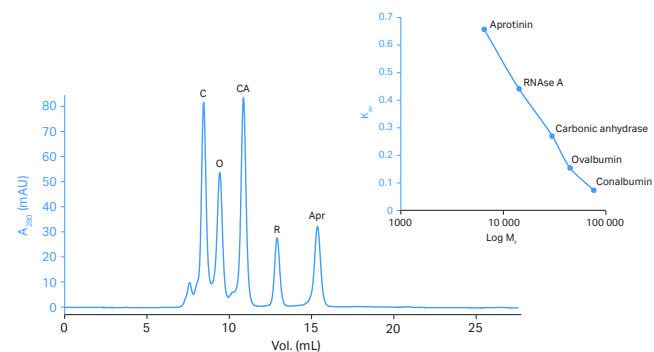
Typical calibration results from chromatographic runs and calculated calibration curves using prepacked Superdex Increase, Superose Increase, and Sephacryl columns are shown in Figures 2 to 11.

The method used for Figures 2 to 11:

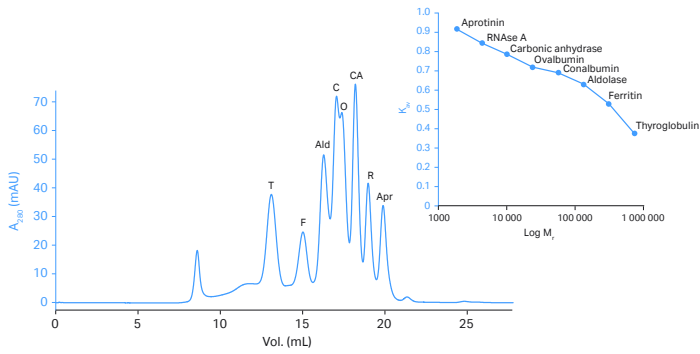
|                       |   |
|-----------------------|---|
| <b>Sample:</b>        | Proteins from Gel Filtration Calibration Kit LMW and HMW: aprotinin (Apr), RNase A (R), carbonic anhydrase (CA), ovalbumin (O), conalbumin (C), aldolase (Ald), ferritin (F), and thyroglobulin (T) |
| <b>Sample volume: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



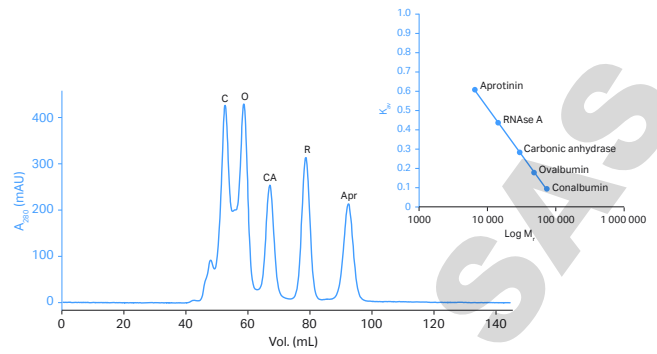
**Fig 2.** Chromatographic separation and calibration curve for the standard proteins on Superdex 200 Increase 10/300 GL column.



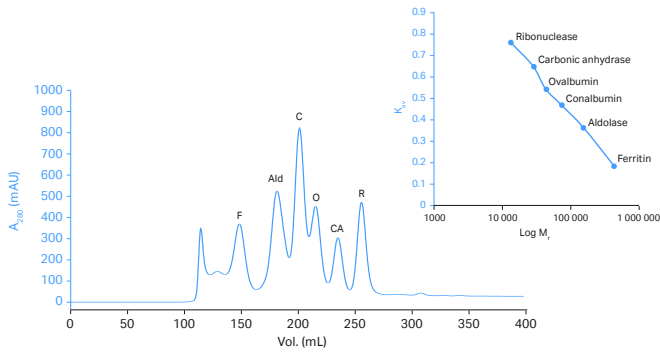
**Fig 3.** Chromatographic separation and calibration curve for the standard proteins on Superdex 75 Increase 10/300 GL column.



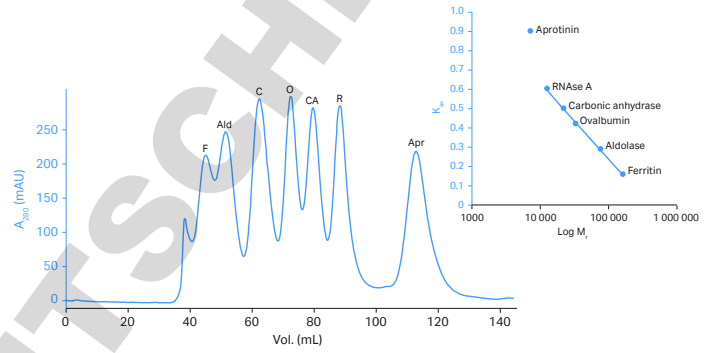
**Fig 4.** Chromatographic separation and calibration curve for the standard proteins on Superose 6 Increase 10/300 GL column. Note: thyroglobulin may be excluded from the calculation of  $K'_{av}$  due to nonlinear behavior of thyroglobulin on this column. Thyroglobulin may however, be included in a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



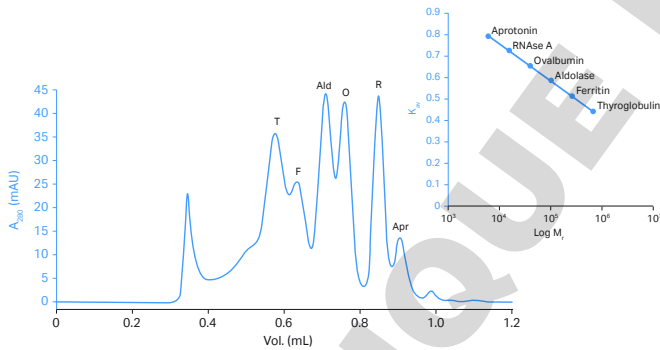
**Fig 8.** Chromatographic separation and calibration curve for the standard proteins on HiLoad 16/600 Superdex 75 pg column.



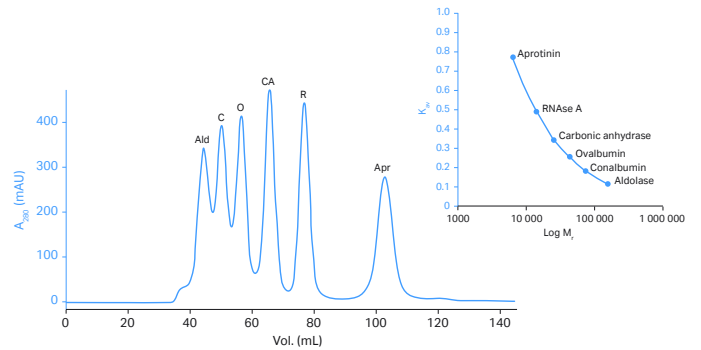
**Fig 5.** Chromatographic separation and calibration curve for the standard proteins on HiLoad™ 26/600 Superdex 200 pg column.



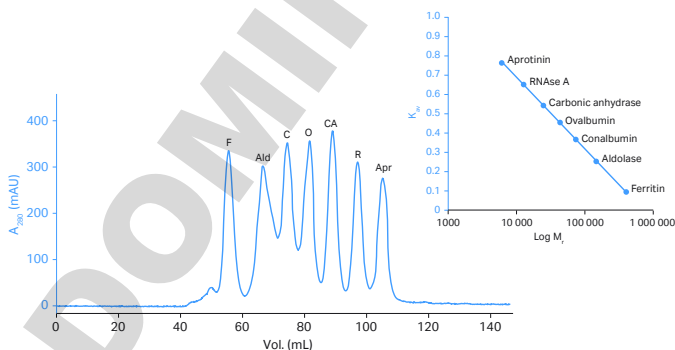
**Fig 9.** Chromatographic separation and calibration curve for the standard proteins on HiPrep™ 16/60 Sephacryl S-300 HR column. Note: aprotinin may be excluded from the calculation of  $K'_{av}$  due to non-linear behavior of aprotinin on this column.



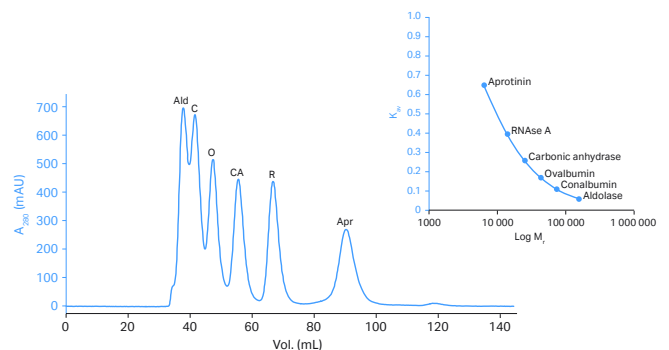
**Fig 6.** Chromatographic separation of the standard proteins on HiLoad 16/600 Superose 6 pg column.



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



**Fig 7.** Chromatographic separation and calibration curve for the standard proteins on HiLoad 16/600 Superdex 200 pg 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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CY13572-07Aug20-DF

