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SEC (Gel filtration) HPLC Protein Standard

Each tube contains protein mixture lyophilized from 80 µL of standard solution.
Total 4 such tubes.

Product Number: **CM92004**

Product Description

CellMosaic's size exclusion chromatography (SEC) or gel filtration protein standard is designed for day-to-day use to check HPLC column performance and the analysis of bioconjugates. This product is a new formulation and is a replacement for both SEC protein standards (Part No. CM92001 and CM92002). The new formulation enables stable lyophilization of the protein mixture and blue dextran without altering the HPLC profile. The lyophilized mixture can be quickly reconstituted in water without forming insoluble particles. The lyophilized product is also more stable for storage and can be shipped at RT.

The product contains six protein standards with molecular weights ranging from 13.7 to 669 kDa, along with blue dextran (2,000 kDa), which serves as a high-molecular-weight reference marker. The broad range of protein molecular weight coverage allows easy identification of small molecules, conjugates, and aggregates in a bioconjugation mixture, such as antibody-drug conjugation, protein, and antibody modification and conjugation. The product has been validated in many of the commonly used SEC columns. CellMosaic routinely uses this product for its internal bioconjugation-related research.

A total of four micro-centrifuge tubes is in a package. Each micro-centrifuge tube contains protein mixture lyophilized from 80 µL of standard solution. The product is ready for use after reconstituting in 80 µL of deionized water.

Application of the Product

- Calibration of gel filtration and size exclusion column
- Determination of the size and molecular mass of proteins
- Analysis of small molecules, bioconjugates, and aggregates

Key Features of the Product

- Lyophilized solid and ready to use for HPLC analysis after dissolving in water
- Mixture of six high-purity proteins (13.7–669 kDa) and blue dextran (2,000 kDa) for broad molecular weight coverage.

Composition

#	Name	MW (kDa)
1	RNase A	13.7
2	Carbonic anhydrase	29
3	Ovalbumin (albumin chicken egg)	43
4	Aldolase (rabbit muscle)	158
5	Ferritin (equine spleen)	440
6	Thyroglobulin	669
7	Blue dextran	2000

Storage/Stability

- The lyophilized product is stable for several years when stored below −20 °C.
- Once reconstituted, the protein standard solution is stable for up to two weeks at room temperature or up to three months at 4 °C, provided evaporation is prevented.

Procedure

1. Take one tube out and warm to ambient temperature.
2. Add 80 µL of deionized water and vortex for 30 seconds to dissolve.
3. Centrifuge briefly to collect the solution at the bottom of the tube. Transfer the solution to an HPLC vial.
4. Inject 8 µL for HPLC analysis (Note: the amount is optimized for a standard SEC HPLC analytical column with a volume of 10-15 mL. For lower or higher column volumes, scale the injection volume accordingly.)

Application Note

Calibration of Protein and Conjugate MW

The approximate MW of an unknown protein or protein conjugate can be determined by comparing its retention time to the protein standard. **Figure 1** gives an example of the SEC profile. For more accurate calculation, an SEC MW standard curve is recommended (**Figure 2**).

Figure 1. SEC HPLC profile of protein standards in a TSKgel BioAssist G3SW$_{xl}$ (7.8 mm x 300 mm, 5 µm, Part No. 0008541). HPLC conditions: Mobile phase: 1× phosphate-buffered saline (PBS), pH 7.4; prepared by a 10-fold dilution of Corning 10× PBS (Corning, Cat. No. 46-013-CM) with deionized water. Flow rate: 0.5 mL/min; column temperature: 25 °C; instrument: Agilent 1100 HPLC system.

#	Name	MW (kDa)	Rt (min)
1	RNase A	13.7	21.397
2	Carbonic anhydrase	29	19.620
3	Ovalbumin (albumin chicken egg)	43	17.433
4	Aldolase (rabbit muscle)	158	15.923
5	Ferritin (equine spleen)	440	13.170
6	Thyroglobulin	669	11.562
7	Blue dextran	2000	11.099

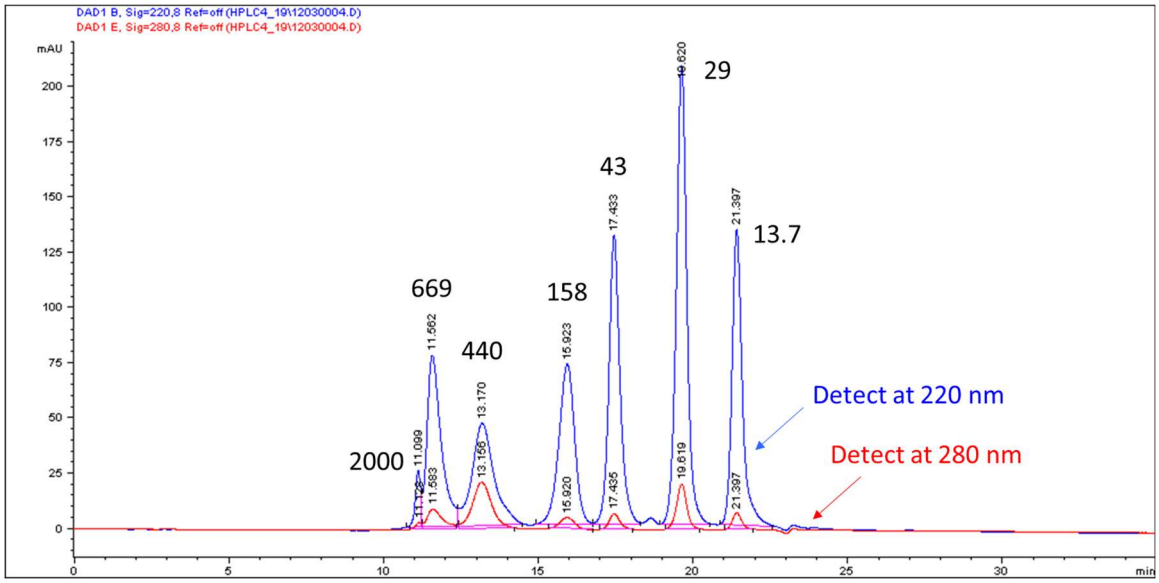
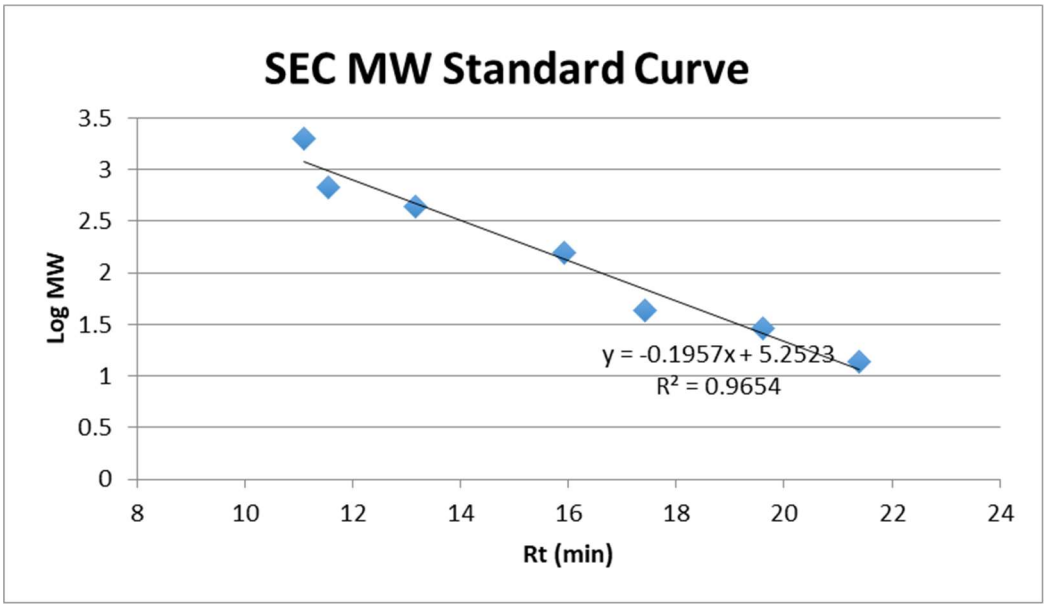


Figure 2. SEC MW standard curve of TSKgel BioAssist G3SW$_{xl}$ (7.8 mm x 300 mm, Part No. 0008541). Data from Figure 1. The logarithm is base 10, and molecular weights are expressed in kDa.



Assessing Column Performance

The protein standard mixture can be used to assess the column performance. The SEC profile of the protein standards may differ from column to column after some use. The quality of the SEC profile will indicate whether the column is good. Split peaks may indicate contamination of the column resin by hydrophobic compounds, suggesting that column cleaning may be necessary.

Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

The information provided in this document and the methods included in this package are for information purposes only. CellMosaic provides no warranty of performance or suitability for the purpose described herein.

Sample data are provided for illustrative and example purposes only and represent a small dataset used to verify kit performance in the CellMosaic laboratory. Information about the chemicals and reagents used in the kit are provided as necessary.

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