



HIC Standard for Column Performance

Each tube contains three proteins and a small molecule mixture in a lyophilized form

Total 2 tubes

Product Number: **CM92007**

Product Description

CellMosaic's Hydrophobic Interaction chromatography (HIC) standard is designed for day-to-day use to check HPLC column performance. The product consists of three proteins and one amino acid with varying surface hydrophobicity. The broad range of hydrophobic coverage allows one to test the performance of HIC column at high range of salt concentration. CellMosaic routinely uses this product for its internal bioconjugation-related research and services.

The product is packaged in a lyophilized form to allow more stable storage and shipment at ambient temperature. The lyophilized protein mixture can be quickly reconstitution in water without any insoluble particles. A total of two micro-centrifuge tubes are in a package. The product is ready for use after reconstituting in 120 μ L of deionized water.

Application of the Product

- Hydrophobic Interaction column performance testing
- HIC HPLC Method development

Key Features of the Product

- Lyophilized solid and ready to use for HPLC analysis after dissolving in water.
- Mixture of 3 high purity proteins and 1 amino acid of varying hydrophobic behavior with broad MW coverage: 155 to 25,600 Da.

Composition

#	Name	MW
1	L-Histidine	155
2	Ribonuclease A	13,700
3	Lysozyme	14,388
4	Chymotrypsinogen A	25,600

Storage/Stability

- Once reconstituted, CellMosaic's protein standard solution is stable at 2-4 °C in a refrigerator for a few days. Self-digested fragments from Chymotrypsinogen A may increase over time. Recommended storage of the product in lyophilized form is below -20 °C.

Usage Procedure

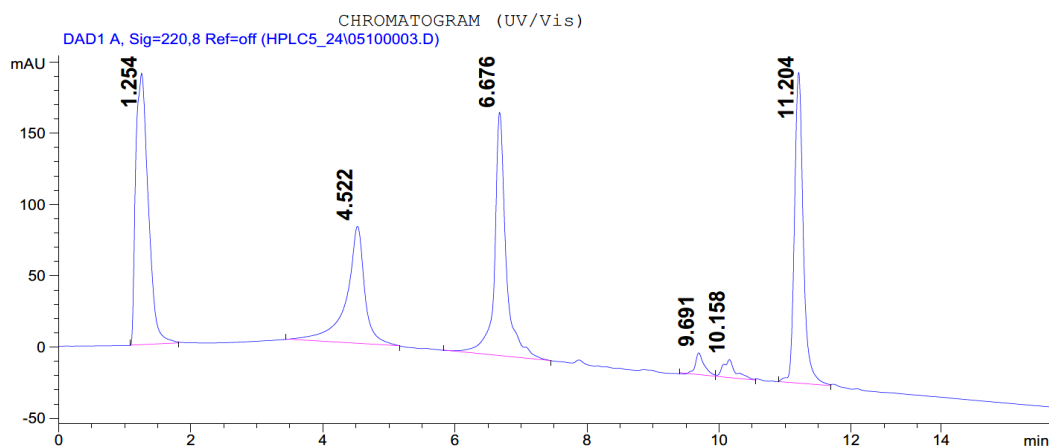
1. Take one tube out and warm to ambient temperature.

2. Add 120 μL of deionized water and vortex for 30 seconds to dissolve.
3. Centrifuge briefly to ensure no liquid is in the cap. Transfer the supernatant to an HPLC vial.
4. Inject 10 μL for HPLC analysis (Note: the amount is optimized for a standard HIC HPLC analytical column with a volume of 1.66 mL – 4.6 mm x 100 mm. For lower or higher column volumes, scale the injection volume accordingly.)

Application Note

The HIC standard can be used to assess the column performance. The HIC profile of the protein standards may differ from column to column after some use. The quality of the HIC profile will indicate whether the column is good. For example, split peaks may indicate column resin-bound hydrophobic molecules and that the column needs cleaning following column manufacturer recommendations.

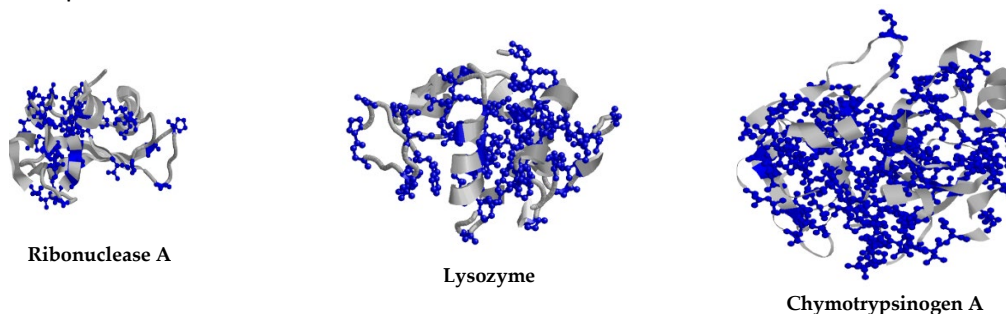
Example 1: Separation of HIC protein standard using a cleaned Waters Protein-Pak™ Hi Res HIC column (4.6 mm x 100 mm, 2.5 μm, Part No. 186007583). HPLC condition: CellMosaic's HIC Buffer set - CM02025 with 15 min gradient at a flow rate of 0.6 mL/min. Instrument: Agilent 1100 HPLC series. HPLC profile is shown in **Figure 1** below.



Note: Self digested fragments from Chymotrypsinogen A are observed at 9.691 and 10.158 min.

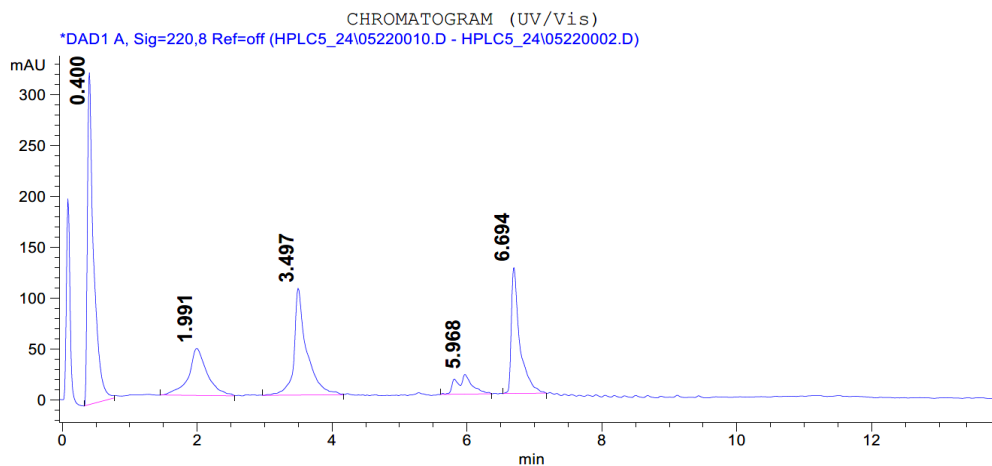
#	Name	MW (Da)	Rt (min)
1	L-Histidine	155	1.254
2	Ribonuclease A	13,700	4.522
3	Lysozyme	14,388	6.676
4	Chymotrypsinogen A	25,600	11.204

Figure 2. Protein structures with hydrophobic sites (patches) on their surface (indicated in blue color) of proteins present in the standard. The higher retention time of the protein, the larger hydrophobic patches shown.



Note: Protein structures with hydrophobic patches were visualized using open source RasWin Molecular Graphics, H. Bernstein®.

Example 2: Separation of HIC protein standard using a new shorter Waters Protein-Pak™ Hi Res HIC column (4.6 mm x 35 mm, 2.5 μm, Part No. 186007582). HPLC condition: CellMosaic's HIC Buffer set - CM02025 with 10 min gradient at a flow rate of 0.8 mL/min. Instrument: Agilent 1100 HPLC series. HPLC profile is shown in **Figure 3** below.



Note: Self digested fragments from Chymotrypsinogen A are observed at 5.968 min.

#	Name	MW (Da)	Rt (min)
1	L-Histidine	155	0.400
2	Ribonuclease A	13,700	1.991
3	Lysozyme	14,388	3.497
4	Chymotrypsinogen A	25,600	6.694

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 product or kit performance in the CellMosaic laboratory. Information about the chemicals and reagents used in the product or kit is provided as necessary.

For Research Use Only. Not for Use in Diagnostic Procedures.

The information in this document is subject to change without notice. CellMosaic assumes no responsibility for any errors that may appear in this document. In no event shall CellMosaic be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple, or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

NOTICE TO PURCHASER: LIMITED LICENSE

The purchase of this product includes a limited, non-transferable license to use this product to practice the labeling methods using the reagents solely for the purchaser's research activities. The license granted herein is personal to the original purchaser and may not be transferred to any other party outside the purchaser's company. No other right or license is conveyed or granted either expressly, by implication or by estoppel, to resell or repackage this product. Further information can be obtained by contacting:

Director of Licensing
c/o CellMosaic, Inc.
10-A Roessler Road, Woburn, MA 01801.
Phone: 781-463-0002
Fax: 781-998-4694
E-mail: info@cellmosaic.com