SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

SigmaProt Intact Protein LC-MS Standard

Catalog Number **MSRT2**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Standards are critical in liquid chromatography-mass spectrometry (LC-MS) analysis of proteins to ensure optimal and consistent system performance before, during, and after sample analysis. They may be used to assess protein elution, to drive intelligent method development, to troubleshoot chromatography, and to demonstrate LC-MS platforms are working properly. Increasing interest in LC-MS analysis of intact proteins[1] requires a suitable protein-based performance standard for LC-MS.

MSRT2 is a mixture of 9 proteins (see Table 1) designed to act as an LC-MS platform standard to assess such properties as:

- LC resolution
- Protein elution profile
- Electrospray source conditions
- Deconvolution parameters

The proteins in MSRT2 were chosen to design a mix with various characteristics, such as:

- a broad range of hydrophobicity
- molecular mass from 5,000 Da to 80,000 Da
- the presence of post-translational modifications, such as sialylated glycans
- ease of electrospray ionization
- quality of deconvoluted mass spectra produced

MSRT2 can be used as a quick test of LC-MS platform performance, comparison of LC gradients and columns, monitoring column and system changes, or to drive method development and establish data processing parameters.

Component

MSRT2 Protein Mix	1 vial

Each vial contains 100 μg each of 9 proteins dried via vacuum centrifugation.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The proteins are soluble in reagents compatible with typical RP-LC-MS workflows. However, to ensure protein recovery is maximized, the recommended solvent is 0.1% trifluoroacetic acid (TFA) in water.

Storage/Stability

The dried protein product is stable for up to 2 years when stored at –20 °C.

Procedure

1. Add 100 μL of 0.1% TFA in water to the vial.
2. Vortex vigorously for 1–2 minutes to yield a solution that is 1 μg/μL of each protein in the mix. This solution can be stored up to 5 days at 4–10 °C or up to 1 month at –20 °C.
3. Equilibrate LC-MS system according to recommended conditions or as desired.
4. Sufficient signal is generally obtained by injecting 5 μL (5 μg load) for a 2.1 mm column, 1 μL (1 μg load) for a 1.0 mm column, or dilute as appropriate for other column configurations. Results are shown in Figure 1.

Reference

1. Young, N.L., and Garcia, B.A., Liquid Chromatography–Mass Spectrometry of Intact Proteins, in *Protein and Peptide Analysis by LC-MS: Experimental Strategies* (Letzel, T., ed.). RSC Chromatography Monographs, Royal Society of Chemistry, (Cambridge, UK: 2011), pp. 38-55.

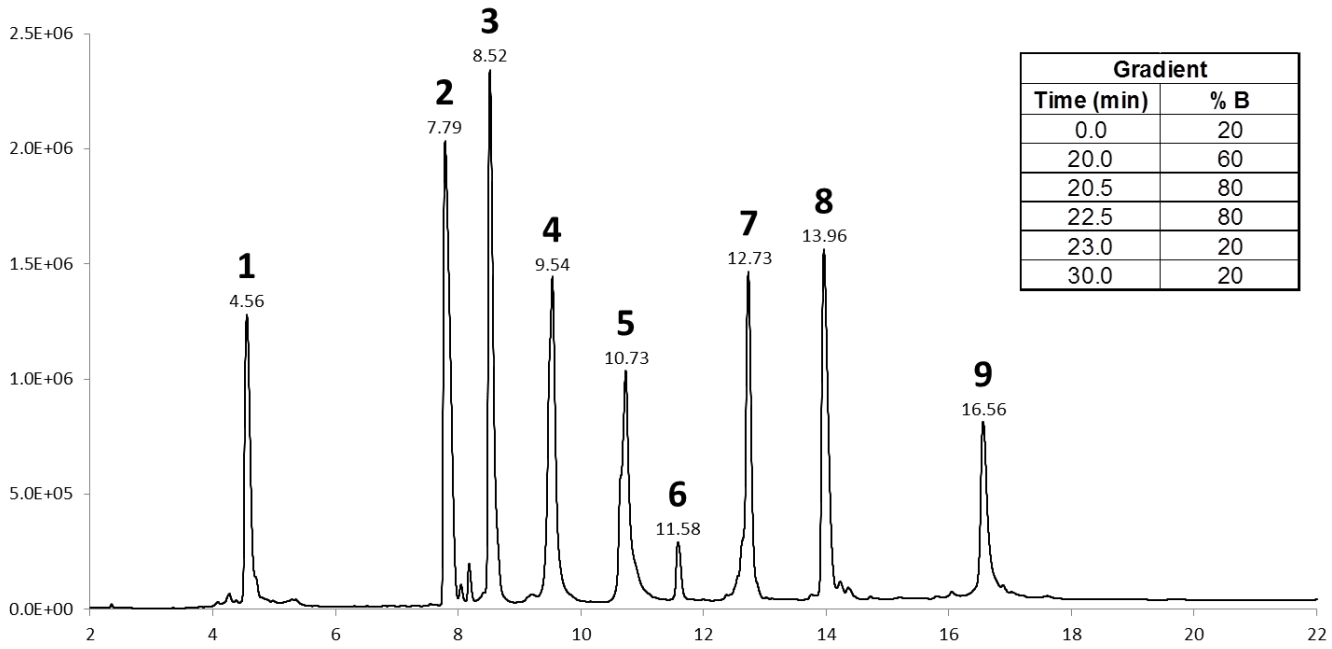
JK,BJC,KR,GCY,MAM 04/17-1

Table 1.
MSRT2 Proteins

			Calculated Mass (Da)	
Protein	**RT Order***	**Additional Information**	**Monoisotopic**	**Average**
Ribonuclease B	**1**	$Man_5GlcNAc_2$	14,889.7	14,899
		$Man_6GlcNAc_2$	15,051.7	15,062
		$Man_7GlcNAc_2$	15,213.8	15,224
		$Man_8GlcNAc_2$	15,375.8	15,386
		$Man_9GlcNAc_2$	15,537.9	15,548
Insulin	**2**		5,803.6	5,808
Lysozyme	**3**		14,295.8	14,305
Transferrin	**4**		79,517.8	79,569
BSA	**5**	BSA	66,386.6	66,430
		BSA-Cysteinylated	66,505.6	66,549
		BSA-Glycated	66,548.6	66,592
Trypsin Inhibitor	**6**	Mature Sequence	20,078.0	20,091
		C-Terminal Leu Truncation	19,965.0	19,978
β-Lactoglobulin A	**7**	β-Lg A	18,351.4	18,363
		β-Lg A-Lactosylated	18,675.5	18,688
Carbonic Anhydrase	**8**	N-Acetylserine	29,006.7	29,025
Lactate Dehydrogenase	**9**	C Chain	36,137.1	36,160

* Confirmed using electrospray ionization following C4 chromatography as described in Figure 1.

Figure 1.
UV_{215} chromatogram of MSRT2 using Waters M-Class ACQUITY UPLC® and Xevo® G2S mass spectrometer. Column = Supelco BIOshell® A400 Protein C4, 150 × 1.0 mm, 3.4 μm (P/N 67045-U) at 65 °C. Flow rate = 70 μL/min with Solvent A = Water, 0.1% Trifluoroacetic Acid, Solvent B = Acetonitrile, 0.1% Trifluoroacetic Acid. Injection = 1 μL for column load of 1 μg each protein.



Gradient	
Time (min)	**% B**
0.0	20
20.0	60
20.5	80
22.5	80
23.0	20
30.0	20