

Product Information

MS RT Calibration Mix

MSRT1

Product Description

Standards are critical in mass spectrometry-based proteomics to ensure optimal and consistent system performance before, during, and after sample analysis. They may be used to assess peptide elution, troubleshoot chromatography, predict retention time, and to demonstrate that the LC-MS platforms are working properly.

MSRT1 is a mixture of 14 isotopically labeled synthetic peptides designed to act as a LC-MS platform standard, containing elements to test:

- LC Resolution
- Peptide elution profile
- LC-MS Platform performance
- Retention time prediction

The peptides in MSRT1 are designed to span the normal elution profile of complex proteomic samples. Also, the peptides have been added in various amounts (based on electrospray response) to have relatively similar responses and be readily observed. MSRT1 can be used as a quick test of LC-MS platform performance, comparison of LC gradients and columns, monitoring column and system changes, or used to predict retention times of peptides across LC-MS platforms.

Reagent

MS RT Calibration Mix: 1 vial

Each vial contains various amounts of 14 isotopically labeled synthetic peptides dried via vacuum centrifugation. Each peptide is isotopically labeled with (¹³C, ¹⁵N) Leucine (+7 Da), (¹³C, ¹⁵N) Lysine (+8 Da), or (¹³C, ¹⁵N) Arginine (+10 Da).

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 peptides are soluble in reagents compatible with proteomic workflows (for example, 0.1–1.0% formic acid). However, peptide recovery is maximized when approximately 20% organic solvent is used to resuspend the dried material. Reconstitution with < 20% CH₃CN may reduce recovery of certain peptides.

Procedure

1. Briefly centrifuge the vial at approximately 10,000 x g to collect the product at the bottom.
2. Add ≥ 10 mL of 20% CH₃CN and 1.0% formic acid.
3. Vortex rigorously for 30–60 seconds.
4. Briefly centrifuge the vial at approximately 10,000 x g to collect the product at the bottom.
5. Add at least 10x the volume used in step 2 of 0.1% formic acid.

Note: If the total volume is 100 mL, then the concentration (based on the amount of GLFIIDD[K] peptide) is 1 pmol/mL. This is the recommended storage concentration. The solution can be stored up to 1 month at 2–8 °C. DO NOT FREEZE after reconstitution.

6. Vortex rigorously for 30–60 seconds.
7. Dilute desired amount of material prepared in step 5 in 0.1% formic acid as suitable for the particular LC-MS analytical system.

Note: It is recommended that steps 6 and 7 be performed on the day of analysis. Some loss of the hydrophobic peptides has been observed upon storage below 1 pmol/mL (based on the amount of GLFIIDD[K] peptide).

Instrument parameters used for selected reaction monitoring analysis (SRM) are provided via a Skyline file and an Excel table, available for download on our website. Users may optimize their specific SRM parameters as necessary.

Peptide #	Peptide Sequence	Amount (pmols)	Monoisotopic MW	iRT *
1	RGDSPASSP[K]	132	1008.5080	-29.6
2	GLV[K]	309	423.2937	-27.4
3	LGGNETQV[R]	81	982.5071	-25.0
4	AEFAEVS[K]	101	887.4480	-9.4
5	SGFSSVSVS[R]	67	1021.5068	4.3
6	ADEGISF[R]	76	903.4325	9.0
7	DISLSDY[K]	120	947.4691	21.0
8	LVNEVTEFA[K]	38	1156.6219	31.4
9	DQGGELLSL[R]	37	1096.5752	38.8
10	GLFIIDD[K]	100	927.5157	54.6
11	LGEYGFQNA[L]	82	1117.5517	61.4
12	YWGVASFLQ[K]	61	1205.6324	84.3
13	TDELQIEGLKEELAYL[R]	350	2176.1291	111.4
14	AVQQPDGLAVLGIFL[K]	46	1675.9752	126.7

Table 1: MSRT1 Peptides

Amino acid in [brackets] denotes site of label incorporation as follows: [K], ¹³C₆ ¹⁵N₂; [R], ¹³C₆ ¹⁵N₄; or [L], ¹³C₆ ¹⁵N₁.

* As determined by C18 chromatography followed by ESI mass spectrometry. iRT values generated using Skyline.¹

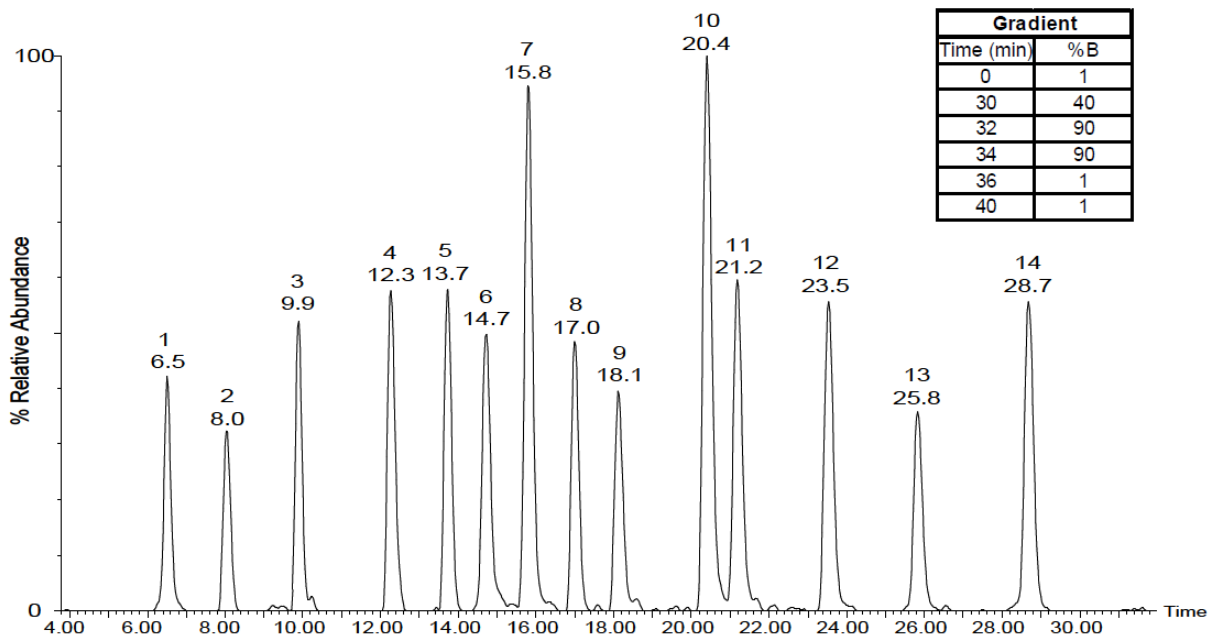


Figure 1: LC-MS Total ion chromatogram of MSRT1 (8 pmols loaded based on the amount of GLFIIDD[K] peptide) using Waters Acquity™ UPLC and LCT mass spectrometer.

Dual Column Ascentis® Express Peptide ES C18

(1 mm I.D. x 15 cm length, 2.7 µm particles, 40 °C) at 90 µL/min

Solvent A: 0.1% formic acid

Solvent B: Acetonitrile with 0.1% formic acid

Reference

1. MacLean, B., et al., Skyline: An Open-Source Document Editor for Creating and Analyzing Targeted Proteomics Experiments. *Bioinformatics*, 26(7), 966-968 (2010).

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MSRT1pis Rev 01/25

