

Pierce Retention Time Calibration Mixture

88320 88321

2281.3

Number	Description
88320	Pierce Retention Time Calibration Mixture, 0.5pmol/μL, 50μL
88321	Pierce Retention Time Calibration Mixture, 5pmol/μL, 200μL

Storage: Upon receipt store at -80°C. Product shipped with dry ice.

Introduction

The Thermo Scientific™ Pierce™ Retention Time Calibration Mixture enables users of liquid chromatography (LC) and mass spectrometry (MS) to confirm correct operation of their LC and MS equipment. Applications of the calibration mixture include optimization of LC parameters, identification of total peptide elution window, and optimization of MS parameters. The calibration mixture can be used with the Thermo Scientific Pinpoint 1.1 software (www.thermoscientific.com/pinpoint) to predict peptide retention times based upon peptide sequence and ease the transfer of methods between LC and MS platforms. The equimolar calibration mixture contains 15 heavy isotope-labeled peptides with each peptide at 0.5pmol/μL (Product No. 88320) or 5pmol/μL (Product No. 88321). The peptide sequences are listed below in Table 1.

Table 1. Peptide sequences, masses and hydrophobicity factors.

<u>Peptide Number</u>	<u>Peptide Sequence*</u>	<u>Mass</u>	<u>Observed Mass, Charge = +2</u>	<u>Hydrophobicity Factor</u>
1	SSAAPPPPPR	985.5220	493.7683	7.56
2	GISNEGQNASIK	1224.6189	613.3167	15.50
3	HVLTSIGEK	990.5589	496.2867	15.52
4	DIPVPKPK	900.5524	451.2834	17.65
5	IGDYAGIK	843.4582	422.7363	19.15
6	TASEFDSAIAQDK	1389.6503	695.8324	25.88
7	SAAGAFGPESLR	1171.5861	586.8003	25.24
8	ELGQSGVDTYLQTK	1545.7766	773.8955	28.37
9	GLILVGGYGTR	1114.6374	558.3259	32.18
10	GILFVGSVSGGEEGAR	1600.8084	801.4115	34.50
11	SFANQPLEVVYSK	1488.7704	745.3924	34.96
12	LTILEELR	995.5890	498.8018	37.30
13	NGFILDGFPR	1144.5905	573.3025	40.42
14	ELASGLSFPVGFK	1358.7326	680.3735	41.18
15	LSSEAPALFQFDLK	1572.8279	787.4212	46.66

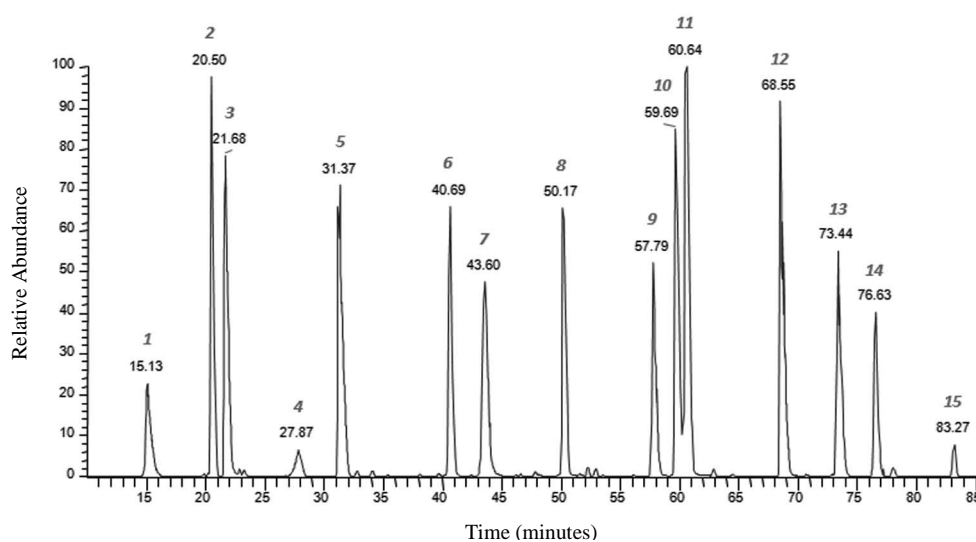
*Amino acids in **bold** are labeled with heavy stable isotopes. Lysine (K) is 8Da heavier and arginine (R) is 10Da heavier.

Important Product Information

- For ease of use and storage of Product No. 88321 (5pmol/μL), prepare aliquots of the peptides in volumes of ≥ 20μL and store each aliquot at -80°C. The peptide mixture is stable for up to one month at 4°C. Product No. 88320 (0.5pmol/μL) can also be aliquoted in volumes of > 20μL, if all of it is not used upon first thawing.
- Use the peptide mixture as a performance evaluation tool for both data-dependent acquisition (DDA) and selective-reaction monitoring (SRM) modes of analysis.
- For SRM methods, not all of the peptides are required for column calibration. Choose at least 8-10 peptides for column calibration. Peptides 9, 13 and 14 may nonspecifically bind to tubes when stored at the lower concentration as in Product No. 88320 (0.5pmol/ml), resulting in a 20-60% lower signal and more variability in their observed intensity.
- Product 88321 is better suited for quantitative dilution curves because of the higher stock peptide concentration.
- All peptides are labeled with a heavy lysine (¹³C₆ ¹⁵N₂, +8Da) or a heavy arginine (¹³C₆ ¹⁵N₄, +10Da) at the C-terminus.
- Please refer to the product web page (link in footer) for additional resources and product information.

Procedure for Use

1. Allow the calibration mixture stock solution, 0.5pmol/μL or 5pmol/μL, to equilibrate to room temperature.
2. Dilute the mixture to 50fmol/μL in LC Buffer A (0.1% formic acid in water) (e.g., for Product No. 88320, combine 20μL peptide mixture plus 180μL LC Buffer A; for Product No. 88321, combine 2μL peptide mixture plus 198μL LC Buffer A).
3. Inject between 250fmol and 1.5pmol per run.
4. For optimal results, use a gradient of 0.25-5% LC Buffer B (0.1% formic acid in acetonitrile) per minute with a 5-25cm column with diameters ranging from 0.075 to 1.0mm.
5. Run each injection in triplicate to ensure proper LC performance.
6. If some peptides fail to resolve, decrease the gradient slope (i.e., lower the change in percentage of LC Buffer B per minute).
7. Once proper chromatographic conditions have been determined, spike the calibration mixture into the analyte and use the peptides to correct for any variability in the injection volumes.



Representative chromatogram. A gradient of 0.25% per minute (Buffer A = 0.1% Formic Acid; Buffer B = 0.1% Formic Acid/99.9% acetonitrile) was used to resolve the Pierce Peptide Retention Time Calibration Mixture. Data was acquired on a Thermo Scientific LTQ Orbitrap-XL Mass Spectrometer using a 0.075 × 200mm Magic™ C18 column (Michrom Bioresources, Inc.) at a flow rate of 300nL per minute. Numbered peaks refer to the corresponding peptides in Table 1.

Troubleshooting

Problem	Possible Cause	Solution
Variable peak intensities	Injector had bubbles	Clean injector with 50% methanol
	Gradient was too steep	Reduce slope of gradient
Peaks are too broad	Gradient was too shallow	Increase slope of gradient
Peaks overlap	Gradient was too steep	Reduce slope of gradient
Some peaks are not detectable	Hydrophobic peptides were retained on the stationary phase	Add up to 5% isopropanol to Buffer B or evaluate alternative stationary phase
Peptide masses are incorrect	MS needed calibration	Calibrate MS

Related Thermo Scientific Products

88322	Pierce LTQ ESI Positive Ion Calibration Solution, 10mL
88323	Pierce LTQ Velos ESI Positive Ion Calibration Solution, 10mL
88324	Pierce ESI Negative Ion Calibration Solution, 10mL
88325	Pierce Triple Quadrupole Calibration Solution, 10mL
88326	Pierce Reserpine Standard for LC-MS, 5 × 1mL

General Reference

1. Krokhin OV, Spicer V. (2009) Peptide retention standards and hydrophobicity indexes in reversed-phase high-performance liquid chromatography of peptides. *Anal Chem* **81**(22):9522-30.

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