

# Pierce™ LC-MS/MS System Suitability Standard (7 x 5 Mix)

Catalog Number A40010

Doc. Part No. 2162731 Pub. No. MAN0018020 Rev. A.0

 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](http://thermofisher.com/support).

## Product description

The Pierce™ LC-MS/MS System Suitability Standard (7 x 5 Mix) enables users of liquid chromatography (LC) and mass spectrometry (MS) to assess system performance as well as dynamic range of LC-MS/MS systems. Applications of the 7 x 5 mixture include optimization of LC parameters, identification of total peptide elution window, and optimization of MS parameters. The mixture can be used in combination with the Skyline software ([skyline.ms](http://skyline.ms)) to assess dynamic range, linearity, and lower limit of quantitation (LLOQ) for 7 peptide groups. The mixture contains 7 of the 15 peptides found in the Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture (Product No. 88320), each having 5 isotopologue sequences present in a dilution series ranging from 0.5 pmol/μL to 0.3 fmol/μL.

Table 1 Modified peptide sequences.

Peptide Group	Peptide Sequence	Precursor m/z (Isotope Label)
1	GISNEGQNA[+4]S[+4][+7]K[+8]	620.8324++ (4 heavy)
	GISNEGQNA[+4]SI[+7]K[+8]	618.8289++ (3 heavy)
	GISNEGQNASI[+7]K[+8]	616.8253++ (2 heavy)
	GISNEGQNASIK[+8]	613.3168++ (1 heavy)
	GISNEGQNASIK	609.3097++
2	IGDY[+10]A[+4]GI[+7]K[+8]	433.2621++ (4 heavy)
	IGDYA[+4]GI[+7]K[+8]	428.2485++ (3 heavy)
	IGDYAI[+7]K[+8]	426.2449++ (2 heavy)
	IGDYAGIK[+8]	422.7364++ (1 heavy)
	IGDYAGIK	418.7293++
3	TASEFDSA[+4]I[+7]A[+4]QDK[+8]	703.3481++ (4 heavy)
	TASEFDSAI[+7]A[+4]QDK[+8]	701.3446++ (3 heavy)
	TASEFDSAIA[+4]QDK[+8]	697.8360++ (2 heavy)
	TASEFDSAIAQDK[+8]	695.8324++ (1 heavy)
	TASEFDSAIAQDK	691.8253++
4	ELG[+3]QSG[+3]V[+6]DTYL[+7]QTK[+8]	783.4148++ (4 heavy)
	ELGQSGV[+6]DTYL[+7]QTK[+8]	780.4111++ (3 heavy)
	ELGQSGVDTYL[+7]QTK[+8]	777.4042++ (2 heavy)
	ELGQSGVDTYLQTK[+8]	773.8956++ (1 heavy)
	ELGQSGVDTYLQTK	769.8885++
5	SFANQPL[+7]EV[+6]V[+6]YSK[+8]	754.9149++ (4 heavy)
	SFANQPLEV[+6]V[+6]YSK[+8]	751.4063++ (3 heavy)
	SFANQPLEV[+6]YSK[+8]	748.3994++ (2 heavy)
	SFANQPLEVYSK[+8]	745.3925++ (1 heavy)
	SFANQPLEVYSK	741.3854++
6	LTIL[+7]L[+7]EEL[+7]R[+10]	509.3276++ (4 heavy)
	LTIL[+7]EEL[+7]R[+10]	505.8190++ (3 heavy)
	LTILEEL[+7]R[+10]	502.3104++ (2 heavy)
	LTILEELR[+10]	498.8018++ (1 heavy)
	LTILEELR	493.7977++
7	ELASGLSFP[+6]V[+6]GF[+10]K[+8]	691.4010++ (4 heavy)
	ELASGLSFPV[+6]GF[+10]K[+8]	688.3941++ (3 heavy)
	ELASGLSFPVGF[+10]K[+8]	685.3872++ (2 heavy)
	ELASGLSFPVGFK[+8]	680.3736++ (1 heavy)
	ELASGLSFPVGFK	676.3665++

## Contents

Product	Cat. No. A40010	Storage
Pierce™ LC-MS/MS System Suitability Standard (7 x 5 Mix)	0.5 pmol/μL, 25 μL	Store at -20°C.

### Additional information

- Use the Pierce™ LC-MS/MS System Suitability Standard (7 x 5 Mix) as a performance evaluation standard for both data-dependent acquisition (DDA) and targeted MS (e.g., parallel-reaction monitoring (PRM)) modes of analysis.
- For ease of use and storage of the 7 x 5 mixture (0.5 pmol/μL), prepare aliquots of the peptides in volumes of 5 μL and store each aliquot at -20°C.
- Refer to attachment A for additional information regarding the data acquisition and data analysis of the 7 x 5 system suitability standard mixture for generating calibration curves for the peptide groups and, subsequently, calculating dynamic range, linearity, and the lower limit of quantitation (LLOQ).
- Example calibration curve concentrations generated for each peptide group:

Each Peptide Group (Isotopologue Series)	Concentration on Column (fmol)
4 heavy	200
3 heavy	20
2 heavy	2
1 heavy	0.5
No heavy	0.13

### Materials required but not provided

- Pierce™ Formic Acid, LC-MS Grade (Product No. 28905)
- DMSO, Anhydrous (Product No. D12345)
- Pierce™ Water, LC-MS Grade (Product No. 51140)

### Procedure for LC-MS/MS suitability check (7 x 5 mix)

For instructions to download attachment A and additional files, visit [thermofisher.com/surequantdocs](http://thermofisher.com/surequantdocs) and enter the code to access instrument method and data analysis information.

1. Prepare 7 x 5 diluent (1% formic acid, 5% DMSO) for the 7 x 5 mixture.
  - a. Add 10 μL of Formic Acid (LC-MS Grade) to a 1.5 mL low protein-binding tube or equivalent.
  - b. Add 50 μL of DMSO, Anhydrous and 940 μL of LC-MS-grade water.
  - c. Vortex 10-20 seconds.
2. Thaw Pierce™ LC-MS/MS System Suitability Standard (7 x 5 Mix) for 5-10 minutes at room temperature.
3. Thoroughly vortex the solution for 2 minutes and quick spin by centrifugation.
4. Add 2 μL of Pierce™ LC-MS/MS System Suitability Standard (7 x 5 Mix) and 18 μL of diluent prepared in step 1 to an autosampler vial.
5. Thoroughly mix this solution by repeatedly pipetting.
6. Transfer the autosampler vial to the LC system and inject 4 μL of each replicate sample.
7. Perform data-dependent acquisition (DDA) and subsequent parallel-reaction monitoring (PRM) modes of analysis using the relevant instrument method (web download). See attachment A for more details about the data acquisition and Skyline analysis.
8. Perform data analysis in Skyline using the DDA and PRM Skyline documents. See attachment A for more details about the data analysis using Skyline software.
9. Assess dynamic range, linearity, and LLOQ for each peptide group using the standard curve generated in the Skyline software. See attachment A for more details on data analysis.

RT: 10.00 - 35.00 SM: 7B

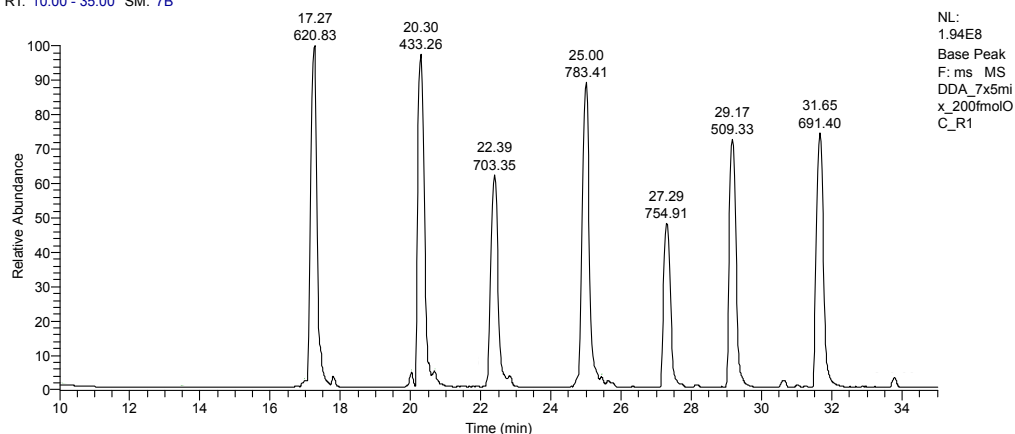


Fig. 1 Example of representative chromatogram for 7 x 5 mixture.

A gradient of 3-35% over 30 minutes (1% increase per minute) (Buffer A = 0.1% Formic Acid; Buffer B = 0.1% Formic Acid/99.9% acetonitrile) was used to resolve the Pierce™ LC-MS/MS System Suitability Standard (7 x 5 Mix). Data was acquired on a Thermo Scientific™ Q Exactive™ HF Mass

Spectrometer using a 0.075 × 150 mm C18 column (Product No. ES800) at a flow rate of 300 nL per minute. The greatest intensity measured for each peak m/z is representative of the "4 heavy" isotope-labeled peptide in Table 1.

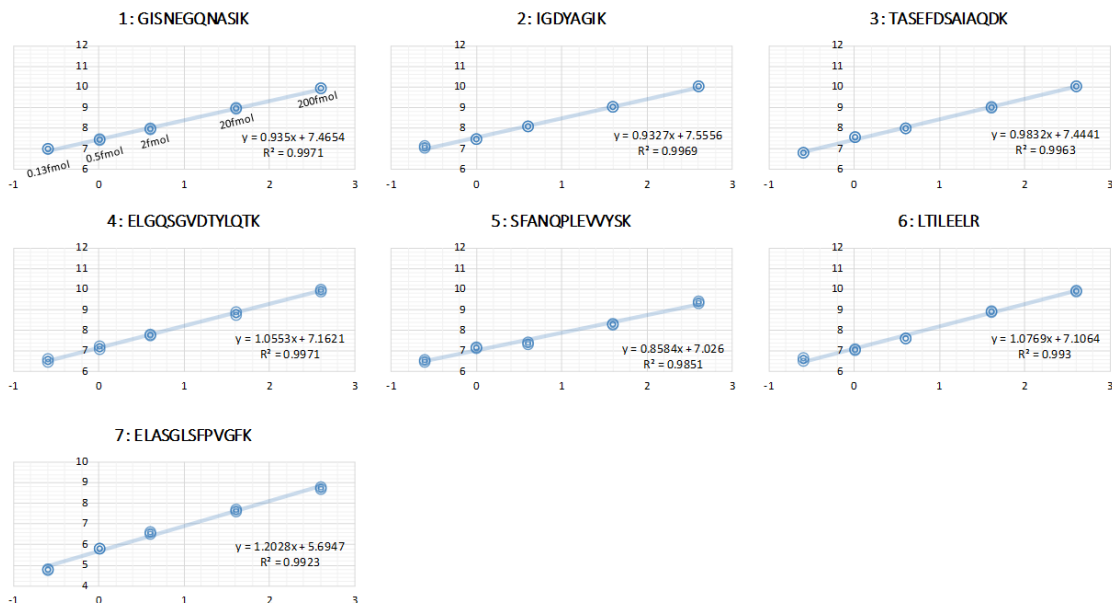


Fig. 2 Calibration curves.

Calibration curves generated for the first 7 peptide groups demonstrate >3 orders for magnitude of dynamic range and linearity ( $R^2$ ) > 0.98.

## Troubleshooting

Observation	Possible cause	Recommended action
Hydrophilic peaks not detected.	Starting percentage of organic mobile phase (%B) was too high.	Decrease starting mobile phase %B to <3%.
	High organic (>5% acetonitrile) was in sample-loading solvent or in sample diluent.	Reduce acetonitrile to <3% in sample-loading solvent and/or in sample solvent.
Hydrophobic peaks are not detected or severely decreased in intensity.	C18 TRAP and/or analytical column was compromised.	Evaluate column with peptide retention time calibration mixture or similar standard. Replace with a new column if necessary.
	Hydrophobic peptides are aggregated or retained on column and/or in tubes.	Ensure low protein-binding tubes are used to prepare peptides, and tubes are vortexed at least 2 minutes.
Peaks are resolved late in the gradient and/or the gradient shifted to the right.	Flow sensor was not calibrated correctly.	Re-calibrate flow sensor module.
	Dead volume in nanoLC system.	Purge nano pumps (A + B) and flush air through the nanoLC system.
Peaks are too broad.	Gradient was too shallow.	Increase the gradient slope.
Peaks overlap.	Gradient was too steep.	Reduce gradient slope.
Peptide masses are incorrect.	MS not calibrated correctly.	Re-calibrate MS.
Some peaks are undetectable.	C18 TRAP and/or analytical column was compromised.	Evaluate column with peptide retention time calibration mixture or similar standard. Replace with a new column if necessary.
	MS front end was dirty.	Run calibration mix to evaluate MS performance and/or clean the front end if necessary.
Variable peak intensities.	Injector had bubbles.	Clean injector with 50% methanol.
	Gradient was too steep.	Reduce gradient slope.

## Related products

Product	Product No.
EASY-Spray™ LC Analytical Column	ES800
Acclaim™ PepMap™ 100 C18 Trap Column, nanoViper™	164564
Pierce™ 0.1% Formic Acid (v/v) in Water, LC-MS Grade	85171
Pierce™ 0.1% Formic Acid (v/v) in Acetonitrile, LC-MS Grade	85175
Pierce™ Acetonitrile (ACN), LC-MS Grade	51101
Pierce™ Water, LC-MS Grade	51140
Pierce™ Trifluoroacetic Acid (TFA)	28904
Pierce™ Formic Acid, LC-MS Grade	28905
DMSO, Anhydrous	D12345
Pierce™ LTQ Velos ESI Positive Ion Calibration Solution	88323

## Limited product warranty

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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](http://thermofisher.com/symbols-definition).

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