SureQuant™ TP53 Panel Relative Quantitation Module

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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**.

Product description

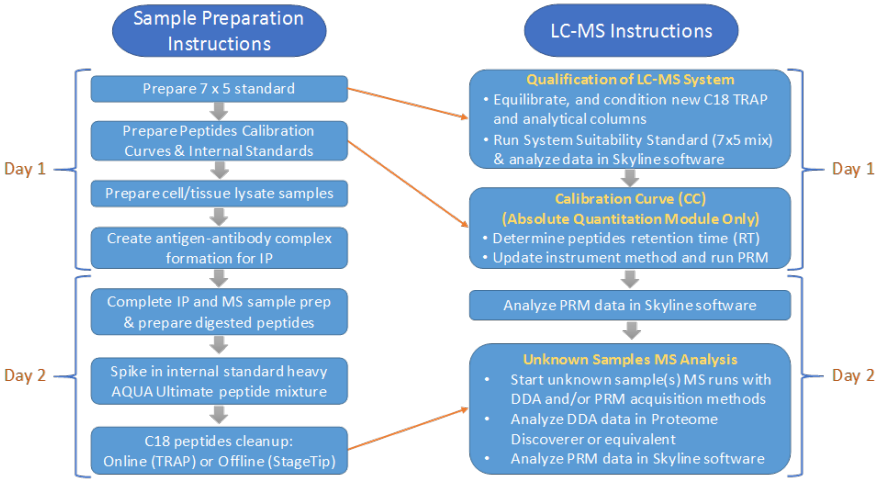
The Thermo Scientific™ SureQuant™ TP53 Panel Relative Quantitation Module enables users to achieve relative quantitation of 4 peptides from TP53 proteins using liquid chromatography (LC) and mass spectrometry (MS). Pierce™ LC-MS/MS System Suitability Standard (7 x 5 Mix) enables the user to assess dynamic range of their nano or capillary flow LC-MS/MS systems before running the unknown samples spiked-in with the internal standard AQUA Ultimate HeavyPeptide mixture and performing quantitative analysis of TP53 target peptides from immune-enriched, digested samples.

Contents

Kit Components	Storage
TP53 Panel AQUA Ultimate HeavyPeptides Mixture, 100 fmol/µL, 100 µL	-20°C
Pierce™ LC-MS/MS System Suitability Standard (7 x 5 Mix), 0.5 pmol/µL, 25 µL	
Peptide Diluent, 5 mL	Room temperature
Low Protein Binding Collection Tubes, 0.6 mL, 50 tubes	

Note: Additional product documents must be downloaded from the website.

Procedure summary



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 parallel-reaction monitoring (PRM) modes of analysis with a LC-MS system.
- For ease of use and storage of TP53 Panel AQUA Ultimate HeavyPeptides (100 fmol/µL), prepare aliquots of the peptides in volumes of 15-20 µL using 0.6 mL low protein-binding tubes provided and store each aliquot at -20°C.
- All AQUA Ultimate HeavyPeptides peptides are labeled with a heavy lysine ($^{13}C_6$ $^{15}N_2$, +8Da) or a heavy arginine ($^{13}C_6$ $^{15}N_4$, +10Da) at the C-terminus.
- Please refer to the attachments A and B (web downloads) for additional information about the data acquisition and data analysis of the system suitability standard, as well as calculations for relative concentration of unknown samples using the Skyline software (at **skyline.ms**).

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

Perform LC-MS/MS system suitability check

Note: Use recommended C18 analytical column (Product No. ES800) and on-line TRAP column (Product No. 164564) for the nanoLC-MS/MS analysis. See attachment A (web download) for the installation and conditioning of C18 columns.

1. Prepare 7 × 5 diluent (1% formic acid, 5% DMSO) for the 7 × 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 7 × 5 system suitability standard and 18 µL of 7 × 5 diluent prepared in step 1 to an autosampler vial.
5. Thoroughly mix this solution by repeatedly pipetting.
6. Transfer the autosampler vial to the nanoLC system and inject 4 µL of replicate samples.
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.

TP53 Panel target peptides relative quantitation from IP-MS samples

1. Thoroughly vortex the AQUA Ultimate HeavyPeptide mixture for 2 minutes. If using frozen stock, thaw the TP53 Panel AQUA Ultimate HeavyPeptide mixture for 15-20 minutes at room temperature before vortexing.
2. Prepare 2 fmol/µL AQUA Ultimate HeavyPeptide internal standard spiked-in solution*:
   a. Pipette 10 µL of AQUA Ultimate HeavyPeptide mixture into a 0.6 mL low protein-binding tube.
   b. Add 490 µL of peptide diluent.
   c. Thoroughly vortex solution for 2 minutes at highest speed setting.

   *The HeavyPeptide internal standard spiked-in solution is used as an internal standard, in which IP-MS samples should be reconstituted as described in the following step.
3. Add 20 µL of AQUA Ultimate HeavyPeptide internal standard spiked-in solution (2 fmol/µL) to each digested and speedvac-dried immunoenriched sample. Store remaining AQUA Ultimate HeavyPeptide internal standard spiked-in solution at -20°C.
4. Vortex each sample for 60 seconds and quickly centrifuge.
5. Remove 12 µL of digested samples with spiked-in AQUA Ultimate HeavyPeptides to a new autosampler vial and place it in autosampler. Inject 5 µL on column for the internal standards spiked-in unknown sample. Store remaining sample at -20°C.
6. Perform data-dependent acquisition (DDA) and subsequent parallel-reaction monitoring (PRM) modes of data acquisition using the relevant LC-MS instrument acquisition method provided with the web download documents for the TP53 Panel. See attachment B for more details about the TP53 Panel DDA and PRM data acquisition methods.

   Note: DDA and PRM LC-MS acquisition methods were optimized using the ES800 C18 column, Easy nanoLC, Dionex™ RSLC nano system, and Q Exactive™- and Fusion™-series MS instruments.
7. Perform DDA data analysis using Thermo Scientific™ Proteome Discoverer™ Software or similar.
8. Perform PRM data analysis in Skyline using the TP53 Panel PRM skyline documents provided with the web download. See attachment B for more details about the TP53 Panel PRM data analysis using Skyline software.
9. Export the analyzed data from Skyline and refer to attachment B for more details on data analysis to calculate the relative concentration of each target peptide per IP from unknown samples.

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 7 × 5 system suitability standard mixture. Replace with new column if necessary.
	Hydrophobic peptides were aggregated or retained on column and/or in tubes.	Ensure that low protein-binding tubes are used to prepare peptides, and that tubes are vortexed at least 2 minutes.
Not all target peptides are identified in the Positive Control Lysate.	Some peptide loss may occur with different C18 sample clean-up methods.	Use on-line C18 Trap column (Product No. 164564) or C18 Spin Tips (Product No. 84850).
Peaks are resolved late in the gradient and/or the gradient shifted to the right.	Flow sensor calibration issue.	Recalibrate flow sensor module.
	Dead volume in nanoLC system.	Purge nano pumps (A + B) and flush air nanoLC system.
Peaks are too broad.	Gradient was too shallow.	Increase slope of gradient.
Peaks overlap.	Gradient was too steep.	Reduce slope of gradient.
Peptide masses are incorrect.	Mass spectrometer needed calibration.	Calibrate mass spectrometer.
Some peaks are not detectable.	C18 TRAP and/or analytical column was compromised.	Evaluate column with 7 × 5 system suitability standard mixture. Replace with new column if necessary.
	Mass spectrometer front end was dirty.	Run calibration mix to evaluate MS performance and/or clean the front end.
Variable peak intensities.	Injector had bubbles.	Clean injector with 50% methanol.
	Gradient was too steep.	Reduce slope of gradient.

Related products

Product	Cat. No.
EASY-Spray™ LC Analytical Column, 75 µm × 150 µm, 3 µm	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™ 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**.