

Details of LC-MS/MS analysis for NP project

liquid chromatography (LC)

instrument	Ultimate 3000 RSLC (Dionex, Idstein, Germany)
trap column	75 μ m inner diameter, packed with 3 μ m C18 particles (Acclaim PepMap100, Thermo Scientific)
analytical column	15 cm analytical column packed with 2 μ m C18 particles (Acclaim PepMap RSLC, Thermo Scientific)
buffer system	binary buffer system consisting of 0.1% acetic acid, 2% ACN (buffer A) and 100% ACN in 0.1% acetic acid (buffer B)
flow rate	300 nl/min
gradient	linear gradient of buffer B from 2% up to 25%
gradient duration	90 min for quantitative proteome analysis (SILAC) 30 min for qualitative proteome analysis (bacterial samples enriched by cell sorting or magnetic capture)
column oven temperature	40°C

mass spectrometry (MS)

instrument	Q Exactive mass spectrometer (Thermo Scientific)
electrospray	via TriVersa NanoMate (Advion Biosciences, Norwich, UK)
operation mode	data-dependent
MS scan resolution	70,000
MS ion target value	3e6
maximum ion injection time for the MS scan	250 ms
selection for MS/MS	10 most abundant isotope patterns with charge ≥ 2 from the survey scan
isolation window	3 m/z
dissociation mode	higher energy collisional dissociation (HCD)
normalized collision energy	27.5%
maximum ion injection time for the MS/MS scans	120 ms
MS/MS ion target value	2e5
dynamic exclusion	40 s

Settings for nLC-MS/MS analysis of cell line comparison project

reversed phase liquid chromatography (RPLC)

instrument	Ultimate 3000 RSLC (Thermo Scientific)
trap column	75 μm inner diameter, packed with 3 μm C18 particles (Acclaim PepMap100, Thermo Scientific)
analytical column	25 cm analytical column packed with 2 μm C18 particles (Acclaim PepMap RSLC, Thermo Scientific)
buffer system	binary buffer system consisting of 0.1% acetic acid, 2% ACN (buffer A) and 100% ACN in 0.1% acetic acid (buffer B)
flow rate	300 nl/min
gradient	linear gradient of buffer B from 2% up to 25%
gradient duration	120 min
column oven temperature	40°C

mass spectrometry (MS)

instrument	Q Exactive mass spectrometer (Thermo Scientific)
electrospray	via TriVersa NanoMate (Advion Biosciences, Norwich, UK)
operation mode	data-dependent

full MS

MS scan resolution	70,000
AGC target	3e6
maximum ion injection time for the MS scan	120 ms
scan range	300 to 1650 m/z
spectra data type	centroid

dd-MS2

resolution	17,500
MS/MS AGC target	2e5
maximum ion injection time for the MS/MS scans	120 ms
selection for MS/MS	10 most abundant isotope patterns with charge ≥ 2 from the survey scan
isolation window	3 m/z
fixed first mass	100 m/z
dissociation mode	higher energy collisional dissociation (HCD)
normalized collision energy	27.5%
dynamic exclusion	30 s