

Supplementary methods. LC-MS^E and search parameters

Description of experiment settings for LC-MS^E analysis and identification of proteins

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|--|---|
| LC-Parameters | |
| LC gradient A: 0.1 % formic acid in water B: 0.1 % formic acid in acetonitrile | 0min-5% B-5m-5%-95m-40%-100m-85%-105m-85%-106m-5%-120m-5% |
| Flow rate | 0.300μL/min |
| Column temperature | 55°C |
| MS^E-Parameters | |
| Lock spray | GluFib precursor 785.8426 m/z |
| Acquisition times | 10-110min |
| Acquisition mode | positive, resolution |
| Mass range | 50-2000Da |
| Capillary voltage | 3kV |
| Sampling cone | 40V |
| Source offset | 80V |
| Source temperature | 80°C |
| Nebuliser gas flow | 6.5 bar |
| Trap collision energy | 4V |
| Transfer collision energy | 2V |
| Protein search parameter | |
| Name of peak list-generating software and release version (number or date) | Progenesis QI v3.0 (Waters Corporation) |
| Name of the search engine and release version (number or date) | MSE built in search engine of Progenesis QI v2.0 (Waters Corporation) |
| Name of database searched and release version/date | Swissprot database release 2022/03 limited to human entries |
| Enzyme specificity considered | Fully tryptic |
| # of missed cleavages permitted | 1 |
| Fixed modification(s) (including residue specificity) | carbamidomethylation of cysteine |
| Variable modification(s) (including residue specificity) | oxidation of methionine |
| Fragment ions per protein | 5 |
| Fragment ions per peptide | 2 |
| Threshold score for accepting protein identification | ≥1 significant peptides |
| Threshold score/E-value for accepting individual MS/MS Spectra | False discovery rate at peptide level <4% |
| software/method used to evaluate site assignment | No PTM reported |