**Combined liquid and mass conditions**

Mass spectrometry scanning parameters:



Liquid elution gradient:

The flow rate was 300nL/min. Buffer A was 0.1%FA aqueous solution and buffer B was 0.1%FA /80% ACN/20% water.



The peptide segment after enzymatic hydrolysis of each sample was separately collected on the machine. The scanning range was set to 350-1250 m/z, and the isolation window was 26 m/z.



DIA mass spectrometry scanning parameters:



The elution gradient of DIA liquid phase:

The flow rate was 300nL/min. Buffer A was 0.1%FA aqueous solution and buffer B was 0.1%FA /80% ACN/20% water.



ectronaut Pulsar software to search and build the library. The main parameters are:



The DIA raw data is processed using the Spectronaut Pulsar software. The key steps are as follows.

1) Open the Analysis module of the Spectronaut Pulsar software and select "+" to create new Analysis;

2) Complete parameter setting step by step and start analysis according to software prompts;

3) After completion of analysis, enter the "Report" module to export quantitative data.



**PRM method export**

1. Import the pre-scanned RAW data into SpectrDive and correct the retention time according to the iRT standard peptide sections. The correction curve is as follows (IRT pre-sweep correction curve):



(2) Set the scheduled method and export:

Scan time window setting:



**Table.1 Reagents and Instruments**

|  |  |  |
| --- | --- | --- |
| **Reagent** | **Manufacturer** | **Model** |
| TMT10 labeling kit | ThermoScientific | 90111LCS |
| TMT6 labeling kit | ThermoScientific | 90066B |
| iTRAQ Labeling Kit | ABSCIEX | 4381663 |
| Plasma de-abundance protein kit (small column) | ThermoFisher | 85165 |
| Plasma de-abundance protein kit (large column) | Millipore | 122642 |
| SDS lysate | Sangon Biotech | P0013G |
| BCA kit | ThermoScientific | 23227 |
| Mass spectrometry water | ThermoScientific | W6-4 |
| Mass spectrometric grade acetonitrile | ThermoScientific | A955-4 |
| PMSF | Amresco | PB0425-5G |
| Disodium hydrogen phosphate dodecahydrate | Sangon Biotech | ST1725-500G |
| Sodium dihydrogen phosphate monohydrate | Sangon Biotech | S0823-100G |
| NaCl | Sangon Biotech | ST1218-1000G |
| 40%Acr-Bis | Sangon Biotech | B546014-0500 |
| Tris-HCl（pH6.8 pH8.8） | Sangon Biotech | B546020-0250 B546019-0250 |
| APS | Sangon Biotech | AB0072-25G |
| TEMED | Sangon Biotech | A100761-0025 |
| Tris | Sangon Biotech | A600194-0500 |
| Glycerin | Sangon Biotech | G0854-500ML |
| BPB | Sangon Biotech | B0449-5G |
| TFA | Sangon Biotech | TS4295-013 |
| IAA | Sangon Biotech | A600539-0005 |
| Urea | Sangon Biotech | UB0148-2000G |
| TEAB | Sangon Biotech | A510932-0500 |
| DTT | Sangon Biotech | A620058-0005 |
| Glycine | Sangon Biotech | 17-1323-01 |
| SDS | Sangon Biotech | 17-1313-01 |
| Phosphoric acid | Sangon Biotech | 10015418 |
| Pancreatin | Sangon Biotech | HLS TRY001C |
| G-250 | Sigma | 27815-25G-F |
| Absolute ethanol | GENERAL-REAGENT | G73537B |
| Isopropanol | GENERAL-REAGENT | G75885B |
| Tris saturated phenol | Solarbio life sciences | T0250 |
| Formic acid | CNW | CAS:64-18-6 |
| Phosphatase inhibitor | Roch | 4906837001 |
| TiO2 | Sangon Biotech | 5020-75000 |
| Sucrose | Sangon Biotech | A100335-0250 |
| EDTA-2Na | Sangon Biotech | A500838-0500 |
| acetone | Sangon Biotech | 40064485 |
| NaCl | Sangon Biotech | A501218-0001 |
| Ammonium acetate | Sangon Biotech | CAS 631-61-8 |
|  |  |  |
| **Instrument** | **Manufacturer** | **Model** |
| Q ExactiveMC-ICP-MS | ThermoFisher |  |
| Q Exactive PlusMC-ICP-MS | ThermoFisher |  |
| Easy-nLC 1200FPLC | ThermoFisher |  |
| Orbitrap FusionMC-ICP-MS | ThermoFisher |  |
| Q Exactive HFMC-ICP-MS | ThermoFisher |  |
| High speed refrigerated centrifuge | Shanghai Lu Xiangyi Centrifuge Instrument Co., Ltd | TGL-16A |
| SDS-PAGEgel electrophoresis | Beijing Liuyi Instrument Factory | DYY-6C |
| Microplate System | Shanghai Kehua Experimental System Co., Ltd. | ST-360 |
| ImageScanner | EPSON | ES-1000G |
| Precision electronic balance | Shanghai Yueping Scientific Instrument Co., Ltd. | FA2004B |
| freeze dryer | SCIENTZ | SCIENTZ-10N |
| High pH separation liquid chromatograph | Agilent | Agilent 1100 series |
| PolySULPHOETHYL A (5 μm, 200 Å) column (200 × 9.4 mm) | PolyLC Inc. | 209SE0502 |
| 96 well SPE desalting column | ThermoFisher | 60309-001 |
| Ultrasonic Cell Disruptor | SCIENTZ | SCIENTZ-IID |
| 3KDultrafiltration tube | Miilipore | UFL900396 |