Tissue lysis and digestion for MS analysis

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Working

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Human Protein Atlas

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ABSTRACT

Here, a workflow for sample preparation and quantification of brain and pancreatic tissue proteins with use of heavy labelled protein standards (QPrESTs) is described. The standards are added before the digestion ensuring high reproducibility and elimination of nuances apearing during proteolytical cleavage of proteins and further steps such as solid phase extraction. QPrESTs are heavy labelled recombinant protein fragments covering regions of proteins with low homology and span over 50-150 aminoacids. They were produced as a part of Human Protein Atlas project in auxotrofic E.coli and individually purified, serving as an excelent resource for MS-based protein quantification.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Methanol (MeOH)	10499560	Fisher Scientific
Acetonitrile (ACN)	10660131	Fischer Scientific
Sodium deoxycholate (SDC)	30970	Sigma Aldrich
Urea	U5378	Sigma Aldrich
Thiourea	T8656	Sigma Aldrich
DL-Dithiothreitol (DTT)	43815	Sigma Aldrich
2-Chloroacetamide (CAA)	22790	Sigma Aldrich
EDTA-free Protease Inhibitor Cocktail	11836170001	Sigma Aldrich
Acetone	34850	Sigma Aldrich
Pierce Trypsin Protease	90057	Thermo Fisher Scientific
Formic acid (FA)	15657520	Sigma Aldrich
Trifluoroacetic acid (TFA)	74564	Sigma Aldrich
Triethylammonium bicarbonate (TEAB)	T7408	Sigma Aldrich
Bio-Rad Protein Assay	5000001	BIO-RAD
Ammonium hydroxide	L13168	Alfa Aesar

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Bio-Rad Protein Assay	5000001	BIO-RAD

MATERIALS TEXT

Buffers were prepared using MilliQ water if not stated differently

Tissue lysis and digestion
Lysis buffer, 1mL (7M Urea, 2M Thiourea, 2% SDC)
m (Urea) = 0.42 g
m (Thiourea) = 0.15 g
m (SDC) = 0.02 g
1/5 tablet of protease inhibitors

300mM DTT, 500 µL
m (DTT) = 23 mg

500mM CAA, 1 mL
m (CAA) = 46.75 mg
Dissolved in 100mM TEAB

100mM TEAB, 1 mL
100 µL 1M TEAB

Solvent A, 100 mL (3% ACN, 0.1% FA)
3 mL 100% ACN
100 µL 100% FA

Solid Phase Extraction using Strong Cation Exchange
Wash Buffer, 1 mL (30% MeOH, 0.1% FA)
300 µL 100% MeOH
1 uL 100% FA

Elution Buffer, 1 mL (30% MeOH, 1.25% NH_4OH)
300 µL 100% MeOH
45 µL 28% NH_4OH

SAFETY WARNINGS

Chloroacetamide (2-chloroacetamide) is a chlorinated organic compound used for alkylating reduced cysteine residues and is suspected of damaging fertility.

Protein extraction

1 Incubate adapter of the Tissue Lyser on dry ice for **00:30:00**

Tissue Lyser LT
QIAGEN 85600
Bead mill

2 Add one 3mm bead to the tissue sample and incubate on dry ice for **00:30:00**

Tungsten Carbide Beads, 3 mm

QIAGEN 69997

Beads for use with Tissue Lyser systems

3 Disrupt the tissue using the Tissue Lyser for 00:02:00

Tissue Lyser LT

QIAGEN 85600

Bead mill

4 Add 250 µl of Lysis Buffer (7M Urea, 2M Thiourea, 2% SDC)

5 Homogenize the tissue using the Tissue Lyser for 00:02:00

Tissue Lyser LT

QIAGEN 85600

Bead mill

6 Centrifuge for 00:30:00 at 20,000 × g at 4 °C

7 Precipitate the proteins with 4 volumes of ice-cold acetone and incubate in -20 °C over night

8 Centrifuge at 20,000 × g at 4 °C for 00:30:00 and discard the supernatant

9 Wash the pellet two times with 400 µl of ice-cold acetone followed by centrifugation at 20,000 × g at 4 °C for

00:10:00 00:10:00

10 Remove the acetone excess and dry the pellet

11 Dissolve the pellet in 200 µl of Pellet Solubilizer (7M Urea, 2M Thiourea)

12 Dilute 8 times with [M]100 Milimolar (mM) TEAB and measure protein concentration using Bio-Rad Protein assay according to the manufacturers instructions

Bio-Rad Protein Assay
by BIO-RAD
Catalog #: 5000001

Protein Digestion

13 Take volume that corresponds to 15 µg of proteins and add QPrESTs mixture

14 Add DTT to a final concentration of [M]10 Milimolar (mM) and incubate 01:00:00 at 30 °C

15 Add CAA to a final concentration of [M]50 Milimolar (mM) and incubate 00:30:00 in the dark at room temperature

16 Add 300 ng of Pierce Trypsin protease and incubate over night at 37 °C

17 Quench the digestion with TFA to a final concentration of 0.5% (v/v)

Solid phase extraction using Strong Cation Exchange

18 Prepare one StageTip per sample by inserting 3 layers of Empore Cation Extraction Discs in a 200uL tip

Empore Discs
47mm cation extraction disks
3M 2251-1

19 Activate membrane with 50 µl 100% MeOH, spin at 1000 x g for 00:01:00

20 Equilibrate StageTip with 50 µl Wash Buffer (30% MeOH, 0.1% FA), spin at 1000 x g for 00:01:00

21 Apply sample to the StageTip, spin at 1000 x g for 00:01:00

22 Wash two times with 30 µl Wash Buffer (30% MeOH, 0.1% FA), spin at 1000 x g for 00:01:00 00:01:00

23 Elute two times with 20 µl Elution Buffer (30% MeOH, 1.65% NH_4OH), spin at 1000 x g for 00:01:00 00:01:00

24 Vacuum dry for 00:30:00 at 42 °C and store at -20 °C until MS-analysis

25 For MS analysis, dissolve samples in Solvent A and inject 1.75 ug