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Tissue lysis and digestion for MS analysis

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David Kotol[1]

[1]Department of Protein Science, Royal Institute of Technology, Stockholm, Sweden

Human Cell Atlas Metho... Human Protein Atlas

David Kotol
Science fo Life Laboratory / KTH - Royal Institute of Techno...

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Protocol status: Working
We use this protocol and it's working

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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.

Materials

MATERIALS

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

STEP MATERIALS

- Bio-Rad Protein Assay **Bio-Rad Laboratories Catalog #**5000001

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

Protocol materials

- 2-Chloroacetamide (CAA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**22790
- Bio-Rad Protein Assay **Bio-Rad Laboratories Catalog #**5000001
- Urea **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**U5378
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- Bio-Rad Protein Assay **Bio-Rad Laboratories Catalog #**5000001

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

Equipment

Tissue Lyser LT	NAME
QIAGEN	BRAND
85600	SKU
https://www.qiagen.com/us/shop/automated-solutions/sample-disruption/tissuelyser-lt/#orderinginformation	LINK
Bead mill	SPECIFICATIONS

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

Equipment

Tungsten Carbide Beads, 3 mm	NAME
QIAGEN	BRAND
69997	SKU
https://www.qiagen.com/se/shop/automated-solutions/accessories/beads/#orderinginformation	LINK
Beads for use with Tissue Lyser systems	SPECIFICATIONS

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

Equipment	
Tissue Lyser LT	NAME
QIAGEN	BRAND
85600	SKU
https://www.qiagen.com/us/shop/automated-solutions/sample-disruption/tissuelyser-lt/#orderinginformation	LINK
Bead mill	SPECIFICATIONS

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

Equipment

Tissue Lyser LT	NAME
QIAGEN	BRAND
85600	SKU
https://www.qiagen.com/us/shop/automated-solutions/sample-disruption/tissuelyser-lt/#orderinginformation	LINK
Bead mill	SPECIFICATIONS

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 millimolar (mM) TEAB and measure protein concentration using Bio-Rad Protein assay according to the manufacturers instructions

Bio-Rad Protein Assay **Bio-Rad Laboratories 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 millimolar (mM) and incubate 01:00:00 at 30 °C

15 Add CAA to a final concentration of [M] 50 millimolar (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

Equipment

Empore Discs	NAME
47mm cation extraction disks	TYPE
3M	BRAND
2251-1	SKU
https://www.sterlitech.com/empore-disks-cation-exchange-sr-2251-1.html	LINK

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