



Feb 25, 2019

Working

Tissue lysis and digestion for MS analysis

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Human Protein Atlas



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.

CATALOG #

VENDOR

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

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NAME

INVALE	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 Y	CATALOG #	VENDOR ~
Bio-Rad Protein Assay	5000001	BIO-RAD

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 gm (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)

 $300~\mu L~100\%~MeOH$

1 uL 100% FA

Elution Buffer, 1 mL (30% MeOH, 1.25% NH₄OH)

300 µL 100% MeOH

 $45~\mu L$ $28\%~NH_4OH$

SAFETY WARNINGS

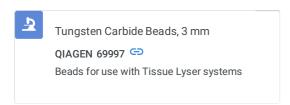
Chloroacetamide (2-chloroacetamide) is a chlorinated organic compound used for alkylating reduced cysteine residues and is suspected of damaging fertility.

Protein extraction

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



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



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



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



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

Precipitate the proteins with 4 volumes of ice-cold acetone and incubate in 8 -20 °C over night

8 Centrifuge at 20,000 \times g at 8 4 °C for \bigcirc 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 3 4 °C for

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- 20 Equilibrate StageTip with 300 μl Wash Buffer (30% MeOH, 0.1% FA), spin at 1000 x g for 300:01:00
- 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 $20 \, \mu$ l Elution Buffer (30% MeOH, 1.65% NH₄OH), spin at 1000 x g for 00:01:00
- 24 Vacuum dry for \bigcirc 00:30:00 at 342 °C and store at 3-20 °C until MS-analysis
- $25\,$ $\,$ For MS analysis, dissolve samples in Solvent A and inject 1.75 ug

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