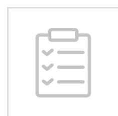

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A membrane-enriched preparation of culture samples for mass spectrometry-based proteomics

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ABSTRACT

Purpose: Preparation of culture samples for mass spectrometry-based proteomics

Principle: Utilizing a membrane-enrichment method of lysing cells and preparing peptides has yielded higher representation of membrane proteins in our mass spectrometry-based proteomic results.Traditional methods do not adequately extract or digest hydrophobic, transmembrane proteins. Particularly, we can now see full expression patterns of proteorhodopsin, something we could not detect using traditional mass spec proteomics prep.This protocol builds on the work of Molloy (2008*) Methods Mol Biol* (doi:10.1007/978-1-60327-064-9_30), Erde et al. (2014) *J. Proteome Res*. (doi:10.1021/pr4010019), and Waldbauer, et al. (2017) Anal. Chem. (doi: 10.1021/acs.analchem.7b02752).

Summary: Pure culture samples were spun down and flash frozen for proteomics.A carbonate extraction protocol was used for membrane enrichment before eFASP.The membrane fraction was enzymatically digested with both chymotrypsin and trypsin and the cytosolic fraction was digested with just trypsin.These samples were then ready to be processed further by *in vitro*isotopic peptide labeling (diDO-IPTL).

MATERIALS TEXT

Equipment
QSonica high power sonicator
Optima MAX-XP Beckman Coulter centrifuge
Regular benchtop centrifuge for Eppendorf tubes
Labconco CentriVap Cold Trap
Sonicator bath
Dry Block
Incubator (37C)
Vortex + Eppendorf tube attachment
10, 20, 200, and 1000 µL pipettes
Tube racks

Materials
10, 20, 200, and 1000 µL tips
Wash solution
Carbonate extraction solution
Polypropylene microfuge tube (Beckman Coulter: 357448)
Exchange buffer
1x LDS buffer
Dithiothreitol (DTT)
Iodoacetamide
Digestion Buffer
Peptide Recovery Buffer
Protein LoBind Tube (Eppendorf: 022431081)
Filtrate tubes and Vivacon 500 (30,000 MWCO HY) concentrator (Sartorius)
Parafilm
Ethyl acetate

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Trifluoroacetic acid (TFA)

Reagents and Solutions
a.Wash Solution: 50 mM Tris-HCl, pH 7.5
b.Carbonate extraction solution: 100 mM sodium carbonate
c.Exchange buffer: 8 M urea, 0.2% (w/v) deoxycholate, 1 M ammonium bicarbonate
d.2x LDS buffer: 0.666 g Tris HCl, 0.682 g Tris Base, 0.800 g LDS, 0.006 g EDTA, 4 g glycerol in 20 mL milliQ
e.Digestion buffer: 50 mM ammonium bicarbonate with 0.2% (w/v) deoxycholate
f.Peptide Recovery Buffer: 50 mM ammonium bicarbonate

Cell Lysis Protocol 3h

1 Cell pellets resuspended in 333 uL wash solution and lysed with QSonica high power sonication (15 min, 1 sec pulse, Ampl 85%)

All samples were previously derived from 4.5 mL pure cultures spun down and flash frozen

2 After sonication, the tubes were centrifuged (2500x*g*, 8 min) to pellet unlysed debris

3 Supernatant was drawn off and added to 830 uL carbonate extraction solution in a polypropylene microfuge tube

It is very important to check that tubes are compatible with ultracentrifuge

4 Shake samples in polypropylene tubes in 4 C for 1 hour

5 After balancing tubes with additional carbonate extraction solution, membrane pellets were spun down in an ultracentrifuge (115,000 x*g*, 1 hr)

6 Draw off supernatant and preserve as “cytosolic” fraction and save pellet as “membrane” fraction.

Cytosolic Fraction Prep 1h

7 Dilute cytosolic fraction samples in 1:1 in exchange + 20 mM DDT. Additional Eppendorf tubes may be necessary.

8 Alkylate cysteine thiols with 60 nM iodoacetamide and incubate at room temperature for an hour in the dark.

Membrane Fraction Prep 3h

9 Disturb membrane pellets with QSonica high power sonication (10 min, 1 sec pulse, Ampl 85%) in 500 uL LDS buffer + 20 mM DTT.

10 Incubate samples at 95 C for 20 minutes

11 Incubate samples at 37 C for 30 minutes

12 Alkylate cysteine thiols with 60 nM iodoacetamide and incubate at room temperature for an hour in the dark.

Enhanced Filter Aided Sample Preparation (eFASP) 3d

13 Mix 50 uL lysate (membrane or cytosolic fraction) with 400 uL exchange buffer on filter unit.

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14 Spin at 14,000 x g for 10 minutes and discard filtrate

15 Repeat steps 13-14 until all lysate is concentrated on filter

16 Wash filter unit 3 times with 200 uL exchange buffer by spinning at 14,000 xg for 10 minutes.Discard filtrate each time.

17 Wash filter 2 times with 200 uL digestion buffer (spin down at 14,000 x g for 10 min)

18 Transfer filter unit to passivated collection tube.

19 Peptide digestion incubation

19.1 For MEMBRANE fraction: add 100 uL digestion buffer and 2 ug chymotrypsin on filter.Incubate overnight at room temperature (seal tubes with parafilm).After overnight incubation, add 2 ug Trypsin and incubate again overnight at room temperature.

19.2 For CYTOSOLIC fraction: add 100 uL digestion buffer and 2 ug trypsin on filter.Incubate overnight at room temperature (seal tubes with parafilm).

20 Centrifuge (14,000 x g for 10 minutes).

KEEP filtrate

21 Add 50 uL peptide recovery buffer and centrifuge for 10 minutes at 14,000 x g.

KEEP filtrate

22 Repeat step 21

23 Add 200 uL ethyl acetate to the filtrate and transfer to LoBind tube.

24 Add 2.5 uL TFA and vortex gently.

25 Nearly fill each tube with ethyl acetate, sonicate for 10 s (note: not high power), centrifuge at 14,000 x g for 10 minutes, then discard upper organic layer.

26 Repeat step 25 two more times.

27 Place sample tubes (uncovered/caps off) in Dry Block set to 60 C for 5 minutes.

28 Freeze sample (-80 C), then centrivap to dryness.

29 Dried samples can now be used for IPTL labeling or can be loaded on mass spec in 2% Acetonitrile, 0.1% formic acid as an unlabeled sample.

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