Apr 21, 2025 Version 1

Sample Preparation for Quantitative Whole Cell Proteome Analysis by Mass Spectrometry V.1

Nature Cell Biology

DOI
https://dx.doi.org/10.17504/protocols.io.n92ld5918v5b/v1

Markus Hartl[1]

[1]Max Perutz Labs – MS Facility

Elias Adriaenssens
Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.n92ld5918v5b/v1

External link: https://doi.org/10.1038/s41556-025-01712-y

Protocol Citation: Markus Hartl 2025. Sample Preparation for Quantitative Whole Cell Proteome Analysis by Mass Spectrometry. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ld5918v5b/v1

Manuscript citation:
Adriaenssens, E., Schaar, S., Cook, A.S.I. *et al.* Reconstitution of BNIP3/NIX-mitophagy initiation reveals hierarchical flexibility of the autophagy machinery. *Nat Cell Biol* **27**, 1272–1287 (2025). https://doi.org/10.1038/s41556-025-01712-y

Protocol status: Working

Created: April 09, 2025

Last Modified: April 21, 2025

Protocol Integer ID: 126559

Keywords: ASAPCRN, sample preparation for quantitative whole cell proteome analysis, quantitative whole cell proteome analysis, mass spectrometry, sample preparation, whole cell

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000350
Marie Skłodowska-Curie MSCA Postdoctoral fellowship
Grant ID: 101062916

Abstract

-

Guidelines

GENERAL NOTE: The protocols are copied from the internal electronic lab notebook and contain specific notes, for example, sample-specific volumes and incubation times. They should be omitted from a general protocol and rather end concentrations and total amounts should be provided where necessary.

Materials

Buffer: 2% SDC [M] 100 millimolar (mM) Tris/HCl pH= pH 8.8 (20 mg SDC in 1 mL)

- PBS
- sodium deoxycholate
- Tris/HCl pH 8.8
- benzonase
- micro BCA assay (Thermo Fisher)
- iodoacetamide
- DTT
- ammonium bicarbonate
- LysC (mass spectrometry grade, FUJIFILM Wako chemicals)
- trypsin (Trypsin Gold, Promega)
- trifluoroacetic acid

Cell Lysis

33m

1 Pre-heat 2% sodium deoxycholate (SDC) [M] 100 millimolar (mM) Tris/HCl pH 8.8 at 95 °C.

2 Add 50 µL hot 2% SDC [M] 100 millimolar (mM) Tris to cell pellet (add more if not soluble).

3 Incubate samples for 00:10:00 at 95 °C. 10m

4 Resuspend thoroughly with pipet, put to 95 °C for 00:03:00. 3m

5 Cool down, add 1 µL benzonase and incubate On ice for 10-30 min.

6 Sonicate 5 cycles 30/30 sec level H in Bioruptor.

7 Let sit for 00:10:00 for extra benzonase treatment. 10m

8 Centrifuge at 15000 x g, 10°C, 00:10:00 to pellet cell debris. 10m

9 Transfer supernatant to fresh 0.6mL tube.

10 Determine protein concentration with micro BCA assay.

Note

NOTE: we use the Micro BCA kit according to manufacturer's instructions https://www.thermofisher.com/order/catalog/product/23235.

In Solution Digest

4h 39m

11 Protein concentration: ~ 5 µg/µL .

12 Transfer 50 µg protein to 0.2mL tube, fill up to 15 µL .

13 Reduction: [M] 10 millimolar (mM) DTT ([M] 250 millimolar (mM) stock) 00:30:00 50 °C . Here we used 0.6 µL of DTT. 30m

14 Alkylation: [M] 20 millimolar (mM) iodoacetamide (IAA) ([M] 500 millimolar (mM) stock = 0.092 g/mL) 00:30:00 Room temperature in the dark. Here we used 0.6 µL of IAA. 30m

15 Quench the remaining iodoacetamide (IAA) with half amount of DTT used in step 14 and incubate for 00:10:00 at Room temperature . Here we used 0.3 µL DTT to quench. 10m

16 Dilute to 1% final SDC concentration with [M] 100 millimolar (mM) Tris pH pH 8.5 . Here we used 15 µL of SDC.

17 Add 1:100 LysC (100 ng/µl aliquot) – incubate at Room temperature for up to 03:00:00 . Here we used 0.5 µL from 1 µg/µL . 3h

18 Add 1:50 Trypsin (100 ng/µl aliquot) – incubate at 37 °C Overnight . Here we used 1 µL from 1 µg/µL . Here, the total volume was 33 µL at this point of the protocol.

19 Add 10% TFA to reach final 1% TFA, vortex, spin down at 14000 rpm, 00:10:00 . For us here, we added 3.3 µL to reach 10% TFA. 10m

20 Transfer supernatant to new tube.

21 Add 10% TFA to reach final 2% TFA, vortex, spin down at 14000 rpm, 00:10:00 . Here we added 3.3 µL , to reach a total volume of 39.6 µL . 10m

22 Load directly onto prepared C18 STAGEtip or transfer to new tube and load 20%. For us here, 20% equals 10 µg → 8 µL .

23 Desalt: Load the peptides on a C18 STAGEtip.

> Note
>
> 3-5 plugs depending on protein amount, 3 plugs for max. 20 µg .

24 Equilibrate:

24.1 100 µL 100% MeOH, spin 2000 rpm, 00:03:00 . 3m

24.2 100 µL 80% ACN, 0.1% TFA, spin 2000 rpm, 00:03:00 . 3m

24.3 100 µL 0.1% TFA, spin 2000 rpm, 00:03:00 . 3m

25 Load sample: spin 1700 rpm .

26 Wash: 100 µL 0.1% TFA → keep flow through (FT) and wash together.

27 Elute: 2x 30 µL 60% ACN, 0.1% TFA in PCR tube.

28 Speedvac to remove ACN and take up sample in 20 µL 0.1% TFA 2% ACN.