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# Sample Preparation for Quantitative Whole Cell Proteome Analysis by Mass Spectrometry V.2

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## Abstract

This protocol was generated from an internal electronic lab notebook and contains experiment specific notes, for example, samplespecific volumes and incubation times. Due to the automatic generation process it might also contain errors and should be interpreted cautiously. An updated protocols will still be uploaded.This protocol was generated from an internal electronic lab notebook and contains experiment specific notes, for example, sample-specific volumes and incubation times. Due to the automatic generation process it might also contain errors and should be interpreted cautiously. An updated protocols will still be uploaded.

## Guidelines

GENERAL NOTE: This protocol was generated from an internal electronic lab notebook and contains experiment specific notes, for example, sample-specific volumes and incubation times. Due to the automatic generation process it might also contain errors and should be interpreted cautiously. An updated protocols will still be uploaded.

## 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

## Safety warnings

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## 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 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 µL ) 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 µL aliquot) – incubate at Room temperature for up to 03:00:00 . Here we used 0.5 µL from 1 µL . 3h

18 Add 1:50 Trypsin ( 100 µL aliquot) – incubate at 37 °C Overnight . Here we used 1 µL from 1 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.