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Efficient protein extraction for proteomics and metaproteomics (also suitable for low biomass samples)

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

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Abstract

This protocol describes an optimized protein extraction method for (meta-) proteomic analyses. It is based on several existing protocols (see references below) that were combined and adapted and is also compatible with low biomass samples.

References:

Erde et al. 2014, J. Proteome Res. 13, 1885-1895
Kleiner et al. 2017, Nat. Comm. 8, Article Number 1558
Kulak et al. 2014, Nat. Methods 11, 319-324
León et al. 2013, Mol. Cell. Proteomics 12, 2992-3005
Masuda 2008, J. Proteome Res. 7, 731-740
Wang et al. 2015, Int. J. Anal. Chem. 2015, Article ID 763969
Wisniewski et al. 2009, Nat. Methods 6, 359-362

Guidelines

This protocol can be used to extract proteins from biological samples for (meta)proteomics. It is an adaptation of existing protocols*. It can also be used for (very) low-input samples.

***References:**

Erde et al. 2014, J. Proteome Res. 13, 1885-1895
Kleiner et al. 2017, Nat. Comm. 8, Article Number 1558
Kulak et al. 2014, Nat. Methods 11, 319-324
León et al. 2013, Mol. Cell. Proteomics 12, 2992-3005
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Materials

MATERIALS

- Triethylammonium bicarbonate buffer (TEAB)
- Water, uHPLC grade
- Sodium deoxycholate (SDC)
- sodium dodecyl sulfate (SDS)
- Dithiothreitol (DTT)

Safety warnings

Wear appropriate safety clothing (lab coat, safety goggles, gloves, mask) at all times. Wear mask especially when handling DTT, SDS and SDC.
Pelleted SDS is safer and more convenient to handle than SDS powder. Please also refer to the respective MSDS sheets.

Before start

Prepare stock solutions
Prepare lysis buffer
Have ice bath ready
Pre-heat thermoshaker (95 °C)

Stock solutions

1 **Sodium deoxycholate (SDC), 10 % (w/v)**

Weigh in a small amount of SDC in a pre-weighed tube. Add appropriate volume of uHPLC water for a 10 % (w/v) SDC solution (e.g. for 0.1 g of SDC, add uHPLC water to a total volume of 1 ml). Gently vortex until the SDC is almost dissolved. Carefully pipet up and down (avoid extensive foaming) until the SDC is completely dissolved. Prepare freshly directly before use.

Sodium dodecyl sulfate (SDS), 20 % (w/v)

Weigh 2 g SDS in a 15 ml tube. Add uHPLC water to a total volume of 10 ml. Gently invert and wait until the SDS is dissolved. Store aliquots in the dark at room temperature.

DTT, 500 mM

Weigh in a small amount of DTT in a pre-weighed tube. Add approriate volume of uHPLC water for a 500 mM solution (e.g. dissolve 38.56 mg DTT in 0.5 ml uHPLC water). Prepare freshly directly before use.

Lysis buffer

2 Prepare lysis buffer according to the pipetting scheme below:

	stock	final concentration	for 1 ml
TEAB	1 M	50 mM	50 µl
SDC	10 %	1%	100 µl
SDS	20 %	4 %	200 µl
DTT	500 mM	100 mM	200 µl
uHPLC water	-	-	450 µl

Mix carefully (avoid extensive foaming). Keep at room temperature to avoid SDS precipitation. Use within one day.

Cell lysis and protein extraction

3 Cell lysis

- Thaw sample fast at room temperature, keep on ice. Add lysis buffer. Sample:lysis buffer ratio should be at least 1:5 (add more lysis buffer, if necessary, e.g. if the sample is too viscous). Alternatively: add lysis buffer directly to thawing samples (depending on the size of the sample). Proceed immediately.

Homogenize sample:

- Use bead-beating, if necessary (e.g. for hard-to-lyse tissue). Choose the bead size and bead-beating parameters (time, speed, repetitions) according to the sample (e.g. 1.4 mm beads for many tissues; 0.1 mm beads for bacterial cells). This step has to be optimized for the respective sample.
- Cool down samples briefly on ice.
- Gently mix and pipet sample up and down, if possible (e.g. for bacterial cell pellets). Stir carefully before pipetting, so that the pellet does not stick in the pipet tip. This step serves to remove the pellet from the tube wall and to break it up into smaller pieces.
- Vortex briefly (approx. 10 s).
- Pipet up and down (approx. 10 times).
- Heat tubes for 5-10 min at 95 °C and 600 rpm in the thermoshaker. (Be careful: If tubes are more than approx. 1/3 full (depending also on the sample), lids can pop open and sample may get lost. Puncture the tube lid with a cannula to avoid overpressure in the tube).
- Briefly cool samples down on ice.
- Place samples in an ultrasonic bath for 5 min (take care that tubes are fully and safely closed).
- Briefly cool down on ice.

Remove cell debris:

- Centrifuge: 10 min 14.000 x g at room temperature, transfer supernatant to new tube (skip this step for very low-input samples).

Excess sample material can now be frozen at -80 °C and re-used later (precipitation will dissolve after thawing; briefly heat the sample, if necessary).

Proceed with respective protocol (e.g. 1D SDS-gel or filter aided sample preparation (FASP, Wisniewski et al. 2009, Nat. Methods 6, 359-362).