

Cell lysis and protein extraction protocol

A. Materials

- Vortex
- Table centrifuge
- Nanodrop or SDS-PAGE system
- 2,0 and 1,5 mL Eppendorf tubes
- Ice bath + heating block
- Glass beads (approx. 100 μm , acid washed)
- TEAB triethylammonium bicarbonate buffer
- NaDOC (Sodium Deoxycholate)
- B-PER buffer
- Ice cold PBS (1x phosphate buffered saline)
- Cold (-20°C) EtOH (keep at least 30 min at -20°C prior use)
- TEAB Resuspension buffer: 50 mM TEAB, 1% (w/w) NaDOC, adjust to pH = 8.0 using HCl.
- B-PER™ Bacterial Protein Extraction Reagent
- Protein precipitation solution: 100% (w/v) Trichloroacetic acid (TCA).

B. Sampling and quenching from cell cultures

EtOH-based protocol (fast quenching)

1. Cell cultures are mixed with 9-times excess of ice cold (-20°C) EtOH and centrifuged at 3,000g for 5 min @ 4°C .
2. Cell pellets are washed twice with ice-cold EtOH and once with ice-cold PBS (briefly vortex, followed by 5 min centrifugation at 3,000g).
3. Pellets are kept frozen at -80°C until further processed (short term storage and transport at -20°C is also possible).

Alternative PBS-based protocol

4. Cell cultures are centrifuged at 3,000g for 5 min at 4°C .
5. Cell pellets are washed 2 times with ice-cold PBS (brief vortex and 5 min centrifugation at 3,000g).

C. Cell lysis and protein extraction

Procedure

1. Transfer 0.2 g glass beads (100 μm) to a 2 mL Eppendorf tube.
2. Mix 0.35 mL of TEAB resuspension buffer and 0.35 mL of B-PER buffer in a 2 mL Eppendorf tube.
3. Add the resuspension solution to a cell pellet of approx. 25 - 50 mg of biomass (wet weight).
4. Vortex until cell pellet is resuspended in the buffer solution.

5. Add the 0.2 g of glass beads to 2 mL Eppendorf tubes.
6. Homogenize the samples using a vortex for 30 seconds. Repeat 5 times, with a break of approx. 30 seconds in between. Place the sample on ice during breaks.
7. Pellet cell debris (and glass beads) at 14,000 × g at 4°C for 10 min.
8. Carefully transfer 75% of the supernatant to a new Eppendorf tube and keep supernatant at 4-8°C until further processed (if processed later than 1 day keep sample frozen at -20°C).

D. Protein precipitation

1. Add 1 volume of TCA stock to 4 volumes of protein sample (e.g. add 250 µL of TCA solution to 1.0 mL of sample). Important: always use 1.5 ml Eppendorf tubes.
2. Incubate for 10 min at 4°C (e.g. keep tube on ice).
3. Spin sample using a table centrifuge at 14K rpm for 5 min.
4. Carefully discard supernatant solution, leaving the protein pellet intact. The pellet is (usually) formed from whitish, fluffy precipitate.
5. Wash pellet with 200 µL of ice cold acetone.
6. Spin sample once more using a table centrifuge at 14K rpm for 5min.
7. Carefully discard supernatant and keep protein pellet for further processing.
8. Repeat steps 5-7 for a total of 2 acetone washes.
9. Leave Eppendorf tube (containing protein precipitate, open to evaporate residual acetone for a few minutes. Avoid trying at high temperatures.
10. Keep protein pellet (in properly closed tube) at 4-8°C until further processing (**e.g. in-solution digestion protocol**).