SCyCode – PROTOCOL

Protein extraction with SDS buffer (vers. 1.0)

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Reference: developed from MPI FASP protocol

MATERIALS

- SDS Buffer: 4% w/v sodium dodecyl sulfate (SDS), in 100 mM tris(hydroxymethyl)aminomethane (Tris)/HCl; pH 8.0 (▲ HAZARDS)

  Optional phosphatase inhibitors (▲ HAZARDS):
  - Glycerol 2-phosphate (G2-P): 5 mM final concentration from 1 M stock solution
  - Sodium fluoride (SF): 5 mM final concentration from 500 mM stock solution
  - Sodium orthovanadate (SOV): 5 mM final concentration from 100 mM stock solution
  - Ethylenediaminetetraacetic acid (EDTA): 10 mM final concentration from 500 mM stock solution; pH 8.0

- Dithiothreitol (DTT): 1 M stock solution
- Iodoacetamide (IAA): 0.55 M stock solution
- Aceton/methanol (MeOH) mixture: 8:1 v/v mixture
- Deanturation buffer (DB): (6 M urea, 2 M thiourea in 10 mM Tris/HCl; pH 7.5)

EQUIPMENT

- Heat block (95 °C)
- Sonifier (micro tip)

PROCEDURE

REQUIREMENTS

This protocol is optimized for the proteome extraction from microorganisms with high percentages of membrane proteins (e.g. cyanobacteria). Protein reduction and alkylation is performed previous to protein precipitation (overnight) and consecutive digestion. Time exposure until protein precipitation is approximately 2½ hours (steps 1-8).

CELL LYSIS AND PROTEIN PRECIPITATION

1. Dissolve cell pellet in SDS buffer (use 1 mL buffer for each 20 - 50 mg cells)
2. Incubation of suspension in heat block **NOTE[1]** (95 °C for 10 min, vortex in between carefully)
3. Chill suspension on ice (2-5 min)
4. DNA comminution by sonification (sonify the suspension for 30 - 300 s until viscosity is watery, Branson Sonifier 250 with micro tip 5 mm; output control 5, duty cycle 40%)
5. Reduction of cysteine disulfide bonds

   (final concentration 10 mM DTT, incubation for 45 min at RT shaking at 650 rpm)
6. Alkylation of reduced cysteine thiol groups

   (final concentration 5.5 mM IAA, incubation for 45 min at RT shaking at 650 rpm, in the dark)
7. Centrifugation of suspension

   (15 min with 3.345 x g for falcons or 12.000 x g for Eppendorf tubes at RT; keep supernatant)
8. Protein precipitation with acetone/MeOH (mix 1 volume of the supernatant with 8+1 sample volumes of ice cold acetone/MeOH mixture; vortex and incubate o.n. at -20 °C)

WASHING AND REHYDRATION OF PROTEINS

9. Centrifugation of precipitated proteins (5 min with 500 - 1000 x g)

10. Washing: resuspend protein pellet in 80% v/v acetone aq. at RT (use about the double sample volume; vortex thoroughly; the pellet should dissociate; repeat for two or more times)

11. Air dry protein pellet ▲ **CRITICAL:** do not dry to completeness (place the tube in the fume hood and evaporate solvents at RT for approximately 10 - 15 min,)

12. Rehydration of proteins in denaturation buffer

NOTES

[1] **- step 2**:10 min of heat treatment did not affect the number of detected S/T/Y phosphorylation events in a validation of the method.

For organisms with low percentages of membrane proteins (e.g. *E. coli*), heat treatment might not be necessary for efficient protein recovery.

HAZARDS

Substance/Buffer	**Hazardous Component**								
SDS buffer	SDS (solid) Tris	+ +	+				+		
SF (NaF)		+		+	+				
SOV (Na_3VO_4)		+							
EDTA		+							
IAA				+	+				
Acetone		+					+		
MeOH				+	+		+		
DB	urea thiourea	+ +		+ 		+ 			
