TCA protein extraction from diatoms V.1

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1 Works for me
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ABSTRACT

Protein extraction from diatoms using TCA

MATERIALS TEXT

3X SDS Laemmli Buffer:

- 240 mM Tris HCl (pH 6.8)
- 6% SDS
- 30% Glycerol
- 2.28 M β-mercaptoethanol
- 0.06% Bromophenol blue

Storerd: short term at 4°C
long term at -20°C

1 Pellet of around 50 ml of culture in exponential phase (2 million of cells)

2 Wash pellet with 1 ml of TCA 20%

3 Centrifuge at maximum speed for 1 minute

4 Pour off the supernatant and dilute in 100 µl of TCA 20%

5 Add Glass-Beads till meniscus and vortex for 7 minutes

6 Add 400 µl of TCA 5% and transfer the supernatant in new eppendorf (1.5 ml)

7 Centrifuge at 3000 rpm for 10 minutes (chiarification phase)

8 Aspire supernatant containing proteins and put in new eppendorf (1.5 ml)

9 (now quickly!)
Add 100 µl of 3X SDS Laemmli Buffer (see Materials) and vortex very well to dissolve

10 Add 50 µl Tris Base and vortex very well to dissolve

11 Boil at 100°C for 3 minutes

12 Centrifuge at maximum speed for 5 minutes

13 Transfer supernatant containing proteins in a new eppendorf

14 Put tubes on ice

15 Stock at -20°C