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TCA protein extraction from diatoms V.2

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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

Storage: 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 μ l of TCA 20%
- 5 Add Glass-Beads till meniscus and vortex for 7 minutes
- 6 Add 400 μ l of TCA 5% and transfer the supernatant in new eppendorf (1.5 ml)
- 7 Centrifuge at 3000 rpm for 10 minutes (clarification phase)
- 8 Pour off supernatant by aspiration
- 9 (now quickly!)
Add 100 μ l of 3X SDS Laemmli Buffer (see Materials) and vortex very well to dissolve
- 10 Add 50 μ l 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



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