TCA protein extraction from diatoms V.2

Anna Santin¹, Antonella Ruggiero¹, Francesco Manfellotto², Mariella Ferrante²
¹Stazione Zoologica Anton Dohrn Napoli - Italy, ²Stazione Zoologica Anton Dohrn

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

Stored: 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 (clarification phase)

8 Pour off supernatant by aspiration
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