**TCA protein extraction from diatoms V.2**

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**ABSTRACT**

Protein extraction from diatoms using TCA

**DOI**

dx.doi.org/10.17504/protocols.io.bc7rizm6

**PROTOCOL CITATION**

Anna Santin, Antonella Ruggiero, Francesco Manfellotto, Mariella Ferrante 2020. TCA protein extraction from diatoms. protocols.io 
https://dx.doi.org/10.17504/protocols.io.bc7rizm6

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**CREATED**

Mar 04, 2020

**LAST MODIFIED**

Mar 04, 2020

**PROTOCOL INTEGER ID**

33745

**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 (chiarification 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