**Lipid analysis protocol Degerhamn 2014-11-28**

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Based on the Folch method (Folch et al, 1957).

Chemicals;

Prepare a 2:1 Chloroform : Methanol solution

Chloroform

0.73 % NaCl solution

Materials;

Freeze dried algae biomass of known quantity.

Falcon tubes

Aluminium foil

Aluminium cups

Glass tubes that can be centrifuged

20 ml syringe

25 mm w/0.2 PTFE syringe filters

**When working with chloroform and organic solvents, work in a fume hood. Inform other people in the lab about what chemicals you are using. Wear gloves, lab coat and safety goggles**.

1. Suspend the pellet in 4.5 ml Chloroform:Methanol 2:1.
2. Vortex the samples.
3. From this point, protect the samples from exposure to light by wrapping them in tinfoil.
4. Sonicate the samples 2 min 50 % capacity using Vibracell VCX 130. Keep samples on ice.
5. Store samples cold and dark 8 °C until next day.
6. Centrifuge the samples. Discard the upper aqueous supernatant (if any) gently without disturbing the lower Chloroform/MeOH supernatant. Collect the Chloroform/ MeOH supernatant in new clean tubes that can be centrifuged.
7. Repeat the extraction process a total 3 times.
8. To the recovered supernatant, add 3.375 ml 0,73% NaCl solution.
9. Final proportions shall be Chloroform:MeOH:H2O 8:4:3.
10. Vortex until homogenous.
11. Centrifuge 10 min 4000 rpm. Two separate phases are formed; one aqueous and one lipid phase.
12. Remove and discard the aqueous upper phase without interfering with the lower lipid phase.
13. Collect the lipid supernatant and filter through a 25 mm syringe filter 0.2 μm PTFE membrane into pre-weighed aluminium cups. Wash the tube and syringe with chloroform.
14. Add a few drops of MeOH to evaporate traces of water.
15. Evaporate the samples in a fume hood at room temperature.
16. When samples appear dry and the chloroform have evaporated, dry the samples in an oven at 60 °C until constant weight.

References;

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