

Extraction of PLFA from wood debris

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Abstract

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Protocol

Lipid extraction

Step 1.

Mixed the extraction solution with the samples by vortexing. The extraction solution is composed of three solvent. They are PBS (6ML,pH 7.4), Methanol (12ML), Choloroform (6ML).



24 ml Additional info: extraction solution

Sonicated

Step 2.

100 Hz

Water bath

Step 3.

37 °C

The supernatent liquid was transfered to a 50-ml separate funnel

Step 4.

Add Chloroform (6ml) and PBS (6ML) to the funnel

Step 5.

briefly shaken

The mixture need to separate overnight at room temperature

Step 6.

Collect the Chloroform phase

Step 7.

Reduced in volume by rotary evaporation and fractionated by chromatography on silicic acid.

Step 8.

Netural lipid and glycolipids were eluted with chloroform and acetone, respectively, and discarded.

Step 9.

☐ AMOUNT

6 ml Additional info: chloroform

☐ AMOUNT

6 ml Additional info: acetone

Collect the polar lipid eluted with methanol

Step 10.

☐ AMOUNT

6 ml Additional info: methanol

Evaporated under a stream of oxygen-free N₂

Step 11.

Alkaline methanolysis

Step 12.

vortex the mixture for 30s

☐ AMOUNT

1 ml Additional info: 0.56% (w/v) KOH in dried methanol

☐ AMOUNT

1 ml Additional info: toluene:methanol (1:1)

Water bath

Step 13.

37°C

Neutralized with 1 mol/l acetic acid

Step 14.

FAMEs extraction

Step 15.

☐ AMOUNT

4 ml Additional info: 2ml one time; chloroform:hexane (1:4)

Dried under oxygen-free N₂

Step 16.