Oct 16, 2019

Fatty acid extraction and derivatisation

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1 Works for me dx.doi.org/10.17504/protocols.io.79jhr4n

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ABSTRACT

This protocol enables direct, one-pot fatty acid extraction and derivatisation of plant and bacterial samples for preparation for GC-MS analysis.
The protocol was established by the Weber lab for plant seedlings and seeds, but was successfully used for cyanobacteria and *E.coli* as well.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Browse, John, Peter J. McCourt, and Christopher R. Somerville. "Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue." Analytical biochemistry 152.1 (1986): 141-145.

GUIDELINES

All steps can be done at RT

MATERIALS TEXT

- C17 internal standard (stock = 1 mg/ml)
- MeOH//3N HCl (CAS: 7647-01-0)
- hexane (CAS: 110-54-3)
- 1% NaCl
- clean glass tubes

SAFETY WARNINGS

acrid, always wear safety goggles and gloves, don't use plastic

BEFORE STARTING

Always use clean glass tubes and avoid using washing detergent

Start

1 samples with 4 oD-units (e.g.: oD = 1, you will need 4 ml culture)

you should make 4 or more replicates

2 centrifuge in a clean glass tube @ 4500 x g
00:10:00

3 discard supernatant

4 Freeze @ -80 ˚C until further use

Extraction solution

5 200 µl C17 internal standard (1 mg/ml hexane) + 9,8 ml MeOH/3N HCl

caution! fill in a beaker and take the needed amount out of this beaker, don't take out directly from original container to avoid contaminating stock solution.
always work with gloves

Extraction

6 add 1 ml extraction solution to samples and blank
For each new batch of samples, include a blank

7 heat @ 90°C
after 5 minutes re-tighten lids of the glass tubes!
01:00:00

8 let cool down @ RT 5m
00:15:00

9 add 1 ml hexane

hexane is very volatile, that's why working quickly is necessary

10 add 1 ml 1% NaCl

11 vortex
00:00:30

12 spin down @ 2000 rpm
00:05:00

13 transfer hexane phase in to GC vial with screw cap

14 dilute samples (10 µl sample + 90 µl hexane)
wash hamilton between samples with hexane

all of these samples can be frozen @ -20 until further analysis

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