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Fatty acid extraction and derivatisation

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Igem Dusseldorf[1]

[1]Heinrich-Heine Universität Düsseldorf

Igem Dusseldorf

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Manuscript citation:
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.

Protocol status: Working
We use this protocol and it's working

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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.

Guidelines

All steps can be done at RT

Materials

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

Troubleshooting

Safety warnings

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

Before start

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
00:15:00
5m

9 add 1 ml hexane

Note

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