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## Fatty acid extraction and derivatisation

## iGEM Dusseldorf1

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



#### 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 HCI (CAS: <u>7647-01-0</u>)
- 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

- 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.

5m

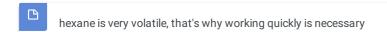
always work with gloves

## 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** 

9 add 1 ml hexane



- 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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