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# Fatty acid extraction and derivatisation

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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.

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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 HCI (CAS: <u>7647-01-0</u>)
- hexane (CAS: <u>110-54-3</u>)
- 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
  - **(5)** 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

**(3)** 00:15:00

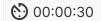
9 add 1 ml hexane 5m



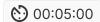
#### Note

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

- 10 add 1 ml 1% NaCl
- 11 vortex



12 spin down @ 2000 rpm



- 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