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

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Fatty acid extraction and derivatisation

Igem Dusseldorf¹

¹Heinrich-Heine Universität Düsseldorf

Igem Dusseldorf

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

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

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 μ l sample + 90 μ l hexane)
wash hamilton between samples with hexane

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