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Fatty acid extraction and derivatisation

iGEM Dusseldorf[1]

[1]Heinrich-Heine Universität Düsseldorf

1 Works for me dx.doi.org/10.17504/protocols.io.767hrhn

iGEM Dusseldorf

ABSTRACT

- C17 internal standard (stock = 1 mg/ml)
- MeOH//3N HCl
- hexane
- 1% NaCl
- clean glass tubes
- always work with glass pipettes or hamiliton

Start

samples with 4 oD-units (e.g.: oD = 1, you will need 4 ml culture)
centrifuge in a clean glass tube for 10 min @ maximum speed
discard supernatant
Freeze @ -80 ˚C until further use

Extraction solution

200 µl C17 internal standard + 9,8 ml MeOH/3N HCl
caution! fill in a beaker and take the needed amount out of this beaker
always work with gloves
pipet C17 internal Std. with hamilton

Extraction

add 1 ml extraction solution to samples and blank
blank: fatty acid standard mix
heat 60 min @ 90°C
let cool down @ RT
add 1 ml hexane
add 1 ml 1% NaCl
vortex for 30 sec
spin down 5 min @ 2000 rpm
transfer hexane phase in to GC vial with screw cap
dilute samples (10 µl sample + 90 µl hexane)
wash hamilton between samples with hexane