Triterpene extraction protocol from Synechocystis sp. PCC6803 and R. capsulatus

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Abstract

In this protocol, the extraction of naturally occurring as well as heterologously synthesized triterpenes in *Synechocystis* and *R. capsulatus* are described

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Before start

Prepare high-quality solvents (opt. LC-MS grade) and add 0.05% BHT (butylated hydroxytoluene) to all solvents (acetone, n-hexan, chloroform/methanol [2:1])

Materials

- Chloroform by Contributed by users
- 100% methanol by Contributed by users
- n-hexane by Contributed by users
- Acetone by Contributed by users
- Beta-Sitosterol by Contributed by users
- NaCl by Contributed by users

Protocol

Step 1.

Collect the volumetric equivalent of $OD_{[750]}$ 20 cellculture, pellet cells and discard the medium supernatant.

Store die pellet in freezer at -80°C in lobind reaction tubes.

Also prepare a solvent control without pellet (mimick pellet with equivalent amount of water (or medium), and proceed exactly as with the “real” samples

Step 2.

Add a total of 10 nmol β-sitosterol (internal standard) to acetone stock and resuspend frozen pellet in 1 ml Acetone and incubate 15 min at 50 °C under gentle agitation

AMOUNT
1 ml Additional info: Acetone
TEMPERATURE
50 °C Additional info: gentle agitation

NOTES
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- do not let it thaw, add acetone directly to frozen Pellet after taking it from the freezer and place immediately to 50°C)

Step 3.

Centrifuge to separate pellet from solvent extract at 1780 rcf for 3 min (flexible), and transfer solvent to new 5 ml reaction tubes

If the pellet is not discolored (white in case of *Rhodobacter*, blue in case of *Synechocystis)*, **definitely repeat extraction step with Acetone** for quantitative analysis and and combine both solutions in the same tube

GOTO
-> go to step #2

Step 4.

Add 1 ml 1 M NaCl to the combined acetone solution and mix

AMOUNT
1 ml Additional info: 1M NaCl

Step 5.

Add 0.9 ml n-Hexane and vortex 30 sec to extract non-polar components to hexane-phase

AMOUNT
0.9 ml Additional info: n-hexan

NOTES
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make sure the phases are vigorously mixed, therefore alternate between vortexing the tube vertically and horizontally, take care to prevent the tube from opening accidentally

Step 6.

Centrifuge shortly for clean phase separation (e.g. 1', 1780 g)

Step 7.

Transfer hexane phase to 2ml reaction tubes.

Repeat the extraction of the pellet with 0.9 ml n-Hexane in order to increase the yield and combine both in the same reaction tube

GOTO
-> go to step #5

NOTES
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take care to transfer the complete hexane phase for quantitative analysis, however avoid contamination with the NaCl-containing lower phase, if you accidentally take too much, you may observe phase separation within your pipet tip, you can carefully let go of the lower few drops (aqueous phase with NaCl) back to the original tube.

Step 8.

Speed vac hexan phase to dryness, as briefly as possible, (at 30°C fast program, about 20 min)

Alternatively use rotation evaporator or N_2-Stream

TEMPERATURE
30 °C Additional info:

Step 9.

Resuspend in 150 µl Chloroform/MeOH (2:1)

AMOUNT
150 µl Additional info: Chloroform/MeOH (2:1)

NOTES
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Prepare a stock of Chloroform/MeOH (2:1) including BHT, do not add MeOH and Chloroform separately

Step 10.

Transfer into HPLC vial with insert

NOTES

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Store extracts at -20 °C