Radiolabeled lipid extraction protocol

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[1]In-house protocol

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1 Works for me
This protocol is published without a DOI.

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ABSTRACT

Radiolabeled - Lipid Extraction Protocol for *Enterococcus faecalis*

PROTOCOL CITATION

Elizabeth Fozo 2020. Radiolabeled lipid extraction protocol. **protocols.io** https://protocols.io/view/radiolabeled-lipid-extraction-protocol-bqewmtfe

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ABSTRACT

Radiolabeled - Lipid Extraction Protocol for *Enterococcus faecalis*

Radiolabeled - Lipid Extraction Protocol for Enterococcus faecalis

1 Grow a large culture of cells (suggest 35 mL per condition) in the lab as normal.

2 Once cells have reached mid-log, harvest 5mL of culture (no fatty acid added yet!) and place cells into a 15mL conical. This is time point zero.

3 Freeze the time point tubes, including time zero, as follow in liquid nitrogen.

4 To a 50 mL conical, add 25 mL of liquid culture.

5 Add in radiolabeled + cold fatty acid mixture (amount to be determined based on target number of µCi [suggest 0.5]); then bring up fatty acid to appropriate concentration), or equivalent volume of ethanol for solvent control.

6 For every time point, harvest 5 mL of the spiked culture. Use filter tip pipettes(1mL each)5x to avoid splashing of hot material.

For each time point, repeat steps 6 and 7

7 Freeze the tube in liquid nitrogen; once all-time points have been collected, proceed at step 8.

8 Add in 5 mL of 1X PBS. Wash two times.

9 After the second wash, tap out the remaining volume of liquid on the pellet onto multiple layers of paper towels; tap in dry locations on the paper towel to avoid saturation through the paper and to avoid collecting hot material on the lip of the conical.

10 Re-suspend pellet in 200uL of 4:1 v/v Ethanol : Water. Use filter tips to avoid aerosols.

11 Boil the suspension in 100*C dry bath for 15 minutes. Vortex about halfway through (at 7.5 min).

12 Centrifuge the boil. Pipet off the supernatant and place into a 2mL glass autosampler vial.

13 Re-suspend the pellet in 100ul of 4:1 v/v Ethanol : Water

14 Boil for an additional 15 minutes. Vortex half way through.

15 Centrifuge the boil. Pipet off the supernatant and place it into the previous 2mL glass autosampler vial.

16 Boil for a final time for 15 minutes. Vortex half way through

17 Centrifuge the boil and pipet the supernatant into the previous 2mL glass autosampler vial.

18 Dry down the samples by leaving the vials on the heat block set to 45*C with the lid off. Drying takes about 24 hours.

19 Re-suspend the dried lipids in 20uL of 2:1 v/v chloroform : methanol.

20 Run on TLC with trimethylamine solvent system.