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# Radiolabeled lipid extraction protocol

Elizabeth Fozo<sup>1</sup><sup>1</sup>In-house protocol

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Works for me

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## ABSTRACT

Radiolabeled - Lipid Extraction Protocol for *Enterococcus faecalis*

## PROTOCOL CITATION

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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  $\mu\text{Ci}$  [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.