OG1RF 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.

Eadewunm

ABSTRACT

Lipid Extraction for *E. faecalis* OG1RF

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Elizabeth Fozo 2020. OG1RF lipid extraction protocol. **protocols.io** https://protocols.io/view/og1rf-lipid-extraction-protocol-bp4smqwe

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ABSTRACT

Lipid Extraction for *E. faecalis* OG1RF

Steps

1 Inoculate 50mL of BHI at O.D. of 0.01 from overnight culture. Spike supplement at OD600 0.25. Incubate for 30 minutes and harvest cells. Spin at 2,739 G for 10 minutes.

2 Wash twice in 25mL 1X PBS. Spin for 5 minutes at 3,000 rpm.

3 Re-suspend pellets in 1mL of PBS

4 Add 100uL of stock lysozyme (100mg/mL – stock made in water) pre-warmed to 37oC. Incubate cells for 20 minutes at 37oC in 50 mL conical.

Meanwhile, add 0.5g glass beads to the screw cap microcentrifuge tube.

5 Add the lysozyme treated cells to screw cap microcentrifuge tube containing 0.5g of glass beads (<100um). Bead beat for two intervals of 1 minute each. Leave on ice at least 30 seconds between cycles.

6 Transfer mixture to 15 mL conical tube (vortex the bead beating tube then pour contents into 15mL conical). Add 1.5mL methanol and 3mL chloroform. (You can add this to the tubes ahead of time.)

7 Then, gently vortex mixture. Centrifuge for 5 minutes.
There will be two layers, separated by a white interface, and glass beads at the bottom of the tube.

8 Using a glass Pasteur pipet – transfer the liquid above the white interface and the liquid below the white interface (leaving behind the beads at the bottom and as much of the interface as possible) to a new 15mL conical.

9 Add 1.5mL of 0.9% NaCl to the new 15mL conical.

10 Gently vortex mixture and centrifuge for 5 minutes.

11 Remove the top layer. Pour interface and bottom layer to glass screw lid tube.

12 Store at -20 until delivery to Mass Spec facility.