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Membrane challenge

Elizabeth Fozo¹¹In-house protocol

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Works for me

This protocol is published without a DOI.



Eadewunm

ABSTRACT

Membrane Stress Challenge Protocol

PROTOCOL CITATION

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<https://protocols.io/view/membrane-challenge-bq9hzm36>



KEYWORDS

Membrane challenge, challenge, membrane, Membrane Stress Challenge Protocol, Membrane Stress Challenge, Stress Challenge Protocol, protocol

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ABSTRACT

Membrane Stress Challenge Protocol

BEFORE STARTING

Note: Put your plates at room temp the day before your experiment

Protocol

- 1 Prepare a 10 ml overnight of OG1RF in BHI. Grow at 37°C

2 Prepare dilution tubes with 0.9% saline - autoclave dilution tubes for 12 minutes

3 In a.m., dilute overnight to b 0.01 in x of BHI + 1.5mM CaCl₂

Prepare BHI +1.5mM CaCl₂: Depending on how much BHI you need, you can take a 250 mL flask, add 100 ml BHI, and 150µl of 1 M CaCl₂. This can be served for blanking and resuspending cells at the end.

Cell growth conditions for long term supplementation

- At the onset of growth (0.01 OD₆₀₀) add your fatty acid supplements.
- Fatty acid supplementation may impact generation time – so start cultures accordingly

4 Once cells about 0.3, harvest 10ml of cell (can spin in plastic)

Cell growth conditions for short-term supplementation

- Grow cells in the absence of fatty acid supplements.
- Once cells reach ~0.225-0.25 spike in fatty acid supplement
- If investigating 2 different fatty acid supplements – grow 25 mL of BHI and then divide into two aliquots with 10 mL – increase the initial quantity of BHI accordingly.
- Incubate for 30 minutes at 37°C

5 Wash cells in 10 ml 1x PBS

6 Resuspend cells in 10 ml BHI = 1.5 mM CaCl₂

7 Transfer 3 ml to a glass tube.

- Plate out Time 0 before adding a membrane stress agent. 10⁻⁵ → 10⁻⁸

8 Add membrane stress agent

- Daptomycin to be 30µg/ml (should be 90µl of 1 mg/ml stock)
- Be sure to check on the concentration you need for your experiment. 30 is just an example.
- SDS to be 0.05% (stock is 10% SDS – 15ul into 3 ml)

9 At times 15, 30, 60 post-membrane stressors, make serial dilutions that can range from 10⁻¹ to 10⁻⁷. Consider a pilot to determine the necessary dilution ranges. Plate the dilutions.

10 Incubate plates overnight at 37°C