Membrane challenge

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[1]In-house protocol

Jan 07, 2021

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

Eadewunm

ABSTRACT

Membrane Stress Challenge Protocol

PROTOCOL CITATION

Elizabeth Fozo 2021. Membrane challenge. **protocols.io**
https://protocols.io/view/membrane-challenge-bq9hmz36

KEYWORDS

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

CREATED

Jan 07, 2021

LAST MODIFIED

Jan 07, 2021

PROTOCOL INTEGER ID

46089

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 $CaCl_2$

Prepare BHI +1.5mM $CaCl_2$: 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 OD_{600}) add your fatty acid supplements.
- Fatty acid supplementation may impact generation time – so start cultures accordingly

4 Once cells about 0.3, harvest10ml 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 cels in 10 ml BHI = 1.5 mM $CaCl_2$

7 Transfer 3 ml to a glass tube.

- Plate out Time 0 before adding a membrane stress agent. $10^{-5} \rightarrow 10^{-8}$

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 overnightat 37°C