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Cerulenin experiment

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[1]In-house protocol

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

Eadewunm

ABSTRACT

Rescue from Cerulenin Inhibition
Based on Saito, et al., 2018

PROTOCOL CITATION

Elizabeth Fozo 2020. Cerulenin experiment. **protocols.io**
https://protocols.io/view/cerulenin-experiment-bpzump6w

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ABSTRACT

Rescue from Cerulenin Inhibition
Based on Saito, et al., 2018

BEFORE STARTING

What you can expect: cerulenin addition with solvent control should inhibit growth completely forE. faecalis. However, you will see a few rounds of division, and then the cells “level-off” at an OD_{600} nm 0.1-0.3.

If the fatty acid can rescue from cerulenin, you should reach a similar OD_{600} nm value at the 24-hour mark as the control tube (cells with no antibiotic). Note that in the case of the specific fatty acid or fatty acid combination, the cells may grow more slowly out of cerulenin inhibition (which is why the 24-hour recording is critical).

Note, as of August 2019, no evidence for suppressors arising from this assay.

Steps

1 Grow strain of interest overnight in 5 ml BHI

2 The next day, set up the following tubes:
Tube with no cerulenin
Tube with cerulenin + solvent control (for fatty acid)
Tube with cerulenin + fatty acid of interest

For current studies, use 5mg/ml of cerulenin (note, can dissolve stock in 10 mg/ml ethanol) and 5ug/ml of fatty acid interested in testing.
Perform in glass tubes with either 5 or 10 mL total volume

3 In am, dilute the strain to be an OD_{600} nm of 0.01 into each of the above tubes. This is time "0"

4 Record the OD_{600} nm at time 2, 4, 6, and 24 hours

5 Graph the results, making sure to add temporal separation in the graphs for the 24 hour time point.