Nov 23, 2020

# Challenge Assay

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[1]In-house protocol

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

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PROTOCOL CITATION

Elizabeth Fozo 2020. Challenge Assay. **protocols.io** https://protocols.io/view/challenge-assay-bpzzmp76



CREATED

Nov 23, 2020

LAST MODIFIED

Nov 23, 2020

PROTOCOL INTEGER ID

44825



## Challenge Assay

1 Have plenty of BHI plates ready. Calculate the number of plates you will need for dilutions (if you expect more killing for a certain strain/condition/time point, dilutions will be lower), label plates accordingly.

2 Prepare plenty of tubes for serial dilutions. Make a solution of 0.9% NaCl, and to dilution tubes, add 900µL. Cap and autoclave.

2.1 Oftentimes, as time progresses, the challenge will do more damage, killing more cells, meaning that for later time points you may not need as many dilution tubes for serial dilutions, though protective fatty acids may increase survival. This will be something to consider before planning your experiment.

3 Prepare overnights of strains.

4 In the morning, dilute cells to an optical density of 0.01 in10 mL supplemented BHI.

4.1 Give an appropriate amount of time for them to reach the same OD; if necessary, start cultures in the incubator at varying times depending on the strain/condition.

4.2 For long-term supplementation, add a supplement to BHI before adding O/N culture.

4.3 For short-term supplementation, grow cells to an OD of ~0.225-0.25 before adding a supplement, then incubate another half-hour before moving on to step 5.

5 Harvest cells – centrifuge; flood with PBS, wash 2x, resuspend in 10 mL BHI.

5.1 Note for daptomycin you need to use calcium for it to be active. So, grow cells, harvest them (with washing steps as required), then resuspend in BHI + 1.5 mM calcium chloride.

6 Take time point zero. Serially dilute culture in dilution tubes containing 0.9% NaCl.

7 Add appropriatevolume/concentration ofchallenge(SDS,daptomycin, bile,etc.)

7.1 For daptomycin, transfer 2.5 mL of cells into a glass tube, and add an appropriate volume of daptomycin to each (we use15 µg/mL as standard).

8 At time points 15, 30, 60 minutes: Serially dilute as in step 6 to appropriate dilutions.

9 Plate dilutions on BHI.

9.1 As you go, plate dilutions on BHI plates. It is okay if you are behind on time to let the culture sit in dilution tubes, but don't squirt dilutions on the plates and allow them to sit for too long as this may cause issues with spreading.

10 Incubate plates overnight. Count plates in the morning, and calculate the log ratio of survivors.