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Cytochrome C Assay

Elizabeth Fozo¹¹In-house protocol

1 Works for me

This protocol is published without a DOI.

Eadewunm

PROTOCOL CITATION

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Steps

- 1 Start ON cultures of a strain of interest.
- 2 The next day, measure 100 mL BHI into each flask to be used.
- 3 Measure OD₆₀₀ of each overnight and calculate how much of your overnight culture you need for OD₆₀₀~ 0.01 in 100mL.

TKO – 20 min – WT – 10 min – DKO

4 Add supplement if doing Long Term exposure or place BHI + 0.01 cells directly into 37°C for a later spike-in

5 Harvest cells at 0.3 if doing Long Term or 0.225-0.25 if doing Spike

Check OD after 2.5 hours.

6 Obtain OD₆₀₀ values for each of your cultures and then calculate how much of your culture is needed to reach an OD₆₀₀ of 7 in 3 mL

1. Formula: $(7 - \text{OD}_{600})(3\text{mL}) = (\text{measured log phase-OD}_{600})(X \text{ mL})$
2. Will need around 60-80mL to achieve this concentration

7 Pipet half the total volume needed into a 50mL conical and centrifuge for ~10 minutes at 3500 RPM. Pour off supernatant. Add the other half of the cell volume. Centrifuge again.

8 Wash the cells with 10 mL of 20mM MOPS buffer* (stock is 1M) by centrifugation for 5 min at 3500 RPM. 20mM MOPS buffer is made in H₂O

C1V1=C2V2 formula to figure out how much stock to make 20mM.

*MOPS-3-(N-Morpholino)propane sulfonic acid resuspend in H₂O – autoclave.

9 Repeat the wash

10 Resuspend the pellet in 3 mL of 20mM MOPS buffer

11 Add 1mg/mL of Cytochrome C (stock is 10mg/mL in H₂O) - vortex

C1V1 = C2V2

12 Incubate for 10 minutes

13 Centrifuge for 10 minutes at 3500 RPM

- 14 Add 2 mL of the supernatant to 4mL plastic cuvettes
- 15 While cytochrome cells are spinning, make standards. To 3 mL of 20mM MOPS buffer add:
 - 0.1 = 30uL of cytochrome
 - 0.2 = 60uL of cytochrome
 - 0.3 = 90uL
 - 0.4 = 120uL
- 16 Use the Cytochrome program ("CYTOCHROME") on the Fozo Lab Spec = OD₅₃₀

Standards listed above cover a range of predetermined mg/mL results, if your results fall outside of this range, you may need to adjust your standards accordingly.