Cytochrome C Assay_small_volume

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[1]In-house protocol

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

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ABSTRACT

Cytochrome C Assay --- Small volume

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ABSTRACT

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BEFORE STARTING

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

Cytochrome C Assay -- Small volume

1 Start ON cultures of the strain of interest
- Usually 5 mL BHI

2 The next day, measure OD_{600} and calculate how much of your overnight culture you need for OD_{600} ~ 0.01
- For each condition, you will need 30mL of cells

3 Add supplement if doing Long Term exposure or place BHI + 0.01 cells directly into 37^{o}C for a later spike-in.

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

5 Obtain OD_{600} values fro each of your cultures and then calculate how much of your culture is needed to reach OD_{600} of 7
- You need 1mL cells at OD_{600} of 7
- Formula: (7 - OD_{600})(1mL) = (measured log phase- OD)(X mL)
- You will need around 25mL to achieve this concentration

6 Use a 50 mL conical and centrifuge your cells for ~ 10 minutes at 3500 RPM. Pour off supernatant

7 Wash the cells with 10 mL of 20mM MOPs buffer*(stock is 1M) by centrifugation for 5 minutes at 3500 RPM. 20 mM MOPS buffer is made in H_2O.
- $C_1V_1 = C_2V_2$ formula to figure out how much stock to make 20mM

8 Repeat the wash

9 Resuspend the pellet in 1 mL of 20 mM MOPS buffer

10 Add 1mg/mL of Cytochrome C (stock is 10mg/mL in HO) - vortex
- $C_1V_1 = C_2V_2$

11 Incubate for 10 minutes at RT

12 Centrifuge for 10 minutes at 3500 RPM

13 Add 800uL of the supernatant to plastic cuvette

14 While cytochrome exposed cells are spinning, make standards. To 1 mL of 20mM MOPS buffer add(remove the appropriate volume of liquid from each 1 mL):
- 0.1 = 10uL of cytochrome
- 0.2 = 20uL of cytochrome
- 0.3 = 30uL of cytochrome

- 0.4 = 40uL of cytochrome

15 Add 800uL of each standard

16 Use the Cytochrome program ("CYTOCHROME") on the Fozo Lab Spec = OD_{530}
- The standard listed above cover a range of predetermined mg/mL results, if your results fall outside the range, you may need to adjust your standards accordingly