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Cytochrome C Assay_small_volume

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Works for me

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ABSTRACT

Cytochrome C Assay --- Small volume

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KEYWORDS

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ABSTRACT

Cytochrome C Assay --- Small volume

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 OD₆₀₀ and calculate how much of your overnight culture you need for OD₆₀₀ ~ 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°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 OD₆₀₀ values from each of your cultures and then calculate how much of your culture is needed to reach OD₆₀₀ of 7
 - You need 1mL cells at OD₆₀₀ of 7
 - Formula: $(7 - OD_{600})(1\text{mL}) = (\text{measured log phase- OD})(X\text{ mL})$
 - 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 H₂O.
 - $C_1V_1 = C_2V_2$ 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 = OD₅₃₀

- 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