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Protocol status: Working
We use this protocol and it's working

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PlatereaderPCAoxidationAssay

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ABSTRACT

Protocol for measuring phenazine-1-carboxylic acid oxidation in a plate reader

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Pre-grow cultures for assay

1 Two days before, streak frozen culture stock on LB agar plate, grow over night at 30 ºC; inoculate liquid cultures for next step with a mixed patch of cells.

2 Grow 5 mL cultures of cells overnight at 30 ºC in LB for 17 hours +/- 10 minutes.

2.1 Depending on the experiment, tubes are either slanted shaking at 250 rpm or standing non-shaking with caps sealed with Parafilm

Prepare cells for assay

3 Wash entire volume into basal medium with no terminal electron acceptor and no PCA (essentially just buffer and salts): 20 mM potassium phosphate buffer (pH 7-7.1); 1 mM sodium sulfate; 10 mM ammonium chloride; and 1× freshwater salt solution (17.1 mM sodium chloride, 1.97 mM magnesium chloride, 0.68 mM calcium chloride, and 6.71 mM potassium chloride)

3.1 In 1 mL aliquots, wash 3x into basal medium by spinning for 2 min at 6000x(g), aspirating with vacuum trap, and resuspending by pipetting.

3.2 Measure OD600 and normalize all cultures to OD600 = 0.2-1, target OD600 = 0.5.

3.3 For slow growing cultures, like *menAubiC-tlKO*, this amounts to spinning down 1 mL of the overnight culture and resuspending it in 250 µL, then adjusting the volume. May need to scale up if inoculating a lot of wells.

4 Bring cultures into the anaerobic chamber.

5 Transfer washed cultures to anoxic microcentrifuge tubes (tubes that have been in chamber for at least three days).

6 Let stand for at least 1 hour. To test that this is fine, can track parallel culture with resazurin to see when it turns pink or clear.

Set up plate (BRAND PureGradeTM S, Cat. No. 781671) inside …

7 Do this as much as possible while cells are incubating in the anoxic chamber

8 Prepare reduced PCA calibration wells

8.1 Prepare 500 µM solution by diluting PCAred stock in basal medium.

8.2 In one row of the plate, prepare calibration (in µM): 250, 200, 175, 150, 125, 100, 75, 50, 25, 10, 5, 0.

This corresponds to the following volumes of the 500 µM PCAred per well (in µL): 100, 80, 70, 60, 50, 40, 30, 20, 10, 4, 2, 0.

8.3 Bring total volume in each well to 200 µL with basal medium

9 Desired concentration of PCA in PCA wells is 200 µM (40 µL of 1 mM stock).

10 Desired concentration of cells is 40 µL of OD600 = 0.5 culture for a target of OD600 = 0.1 in the wells.

11 Other components depend on the experiment: e.g., 2 µL of 1 M sodium nitrate for a 10 mM experimental concentration.

12 Note: all solutions prepared in the same basal medium.

13 Final volume in each well is 200 µL.

14 Order of preparing wells (except calibration): basal medium, then PCA, then TEA, then cells (always last).

Plate reader protocol (BioTek Synergy 4 or HTX)

15 Incubate at 30 ºC throughout experiment. If using HTX model, set the temperature limits +/- 1 ºC to prevent condensation

16 Medium shaking throughout experiment

17 Measure absorbance at 440 and 600 nm (PCA and cells, respectively), as well as fluorescence (360/40 ex and 528/20 em). Test that sensitivity is such that the calibration curve spans the dynamic range (different on each instrument). Include pathlength correction for absorbance measurements.

18 Measure every 5 minutes