Nov 14, 2023

PlatereaderPCAoxidationAssay

DOI
dx.doi.org/10.17504/protocols.io.bp2l6xm6dlqe/v1

Lev Tsypin[1], Dianne K Newman[2], Allen W Chen[2], Scott Saunders[3]
[1]Stanford University; [2]California Institute of Technology; [3]UT Southwestern

Lev Tsypin
Stanford

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DOI: https://dx.doi.org/10.17504/protocols.io.bp2l6xm6dlqe/v1

Protocol Citation: Lev Tsypin, Dianne K Newman, Allen W Chen, Scott Saunders 2023. PlatereaderPCAoxidationAssay. **protocols.io https://dx.doi.org/10.17504/protocols.io.bp2l6xm6dlqe/v1**

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

Created: November 14, 2023

Last Modified: November 14, 2023

Protocol Integer ID: 90896

Keywords: plate reader

Funders Acknowledgements:
NIH
Grant ID: 1R01AI127850-01A1
Doren Family Foundation
NSF GRFP

Abstract

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

Troubleshooting

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 anoxic chamber

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