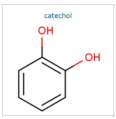

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Measurement of XylE (Catechol 2,3-Dioxygenase) enzyme activity by microplate reader

Ji Gao[1]

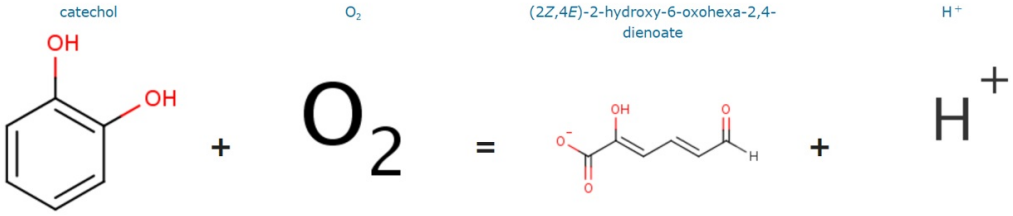
[1]Tsinghua University

1 Works for me dx.doi.org/10.17504/protocols.io.7suhnew

Ji Gao
Tsinghua University

ABSTRACT

Simple measurement of XylE (Catechol 2,3-Dioxygenase) enzyme activity by microplate reader. Catechol 2,3-Dioxygenase can catalyze catechol (1,2-Dihydroxybenzene) to 2-HMS, which has a high absorbance at 377 nm, so we may use photometric mode of microplate reader to measure the concentration of 2-HMS, product of the enzyme.



GUIDELINES

This is part of a tryout for our iGEM project.

The microplate we used is listed here.

Varioskan LUX Multimode Microplate Reader
microplate reader
Thermo Scientific VL0000D2

Perform the measurement at Room temperature or 25 °C .

MATERIALS

NAME	CATALOG #	VENDOR
double distilled water (ddH2O)		
12-Dihydroxybenzene	17253	Adamas-beta
Corning™ 96-Well Clear Bottom Black or White Polystyrene Microplates	07-200-565	Fisher Scientific

SAFETY WARNINGS

Catechol can be hazardous if not properly operated.
Please refer to https://pubchem.ncbi.nlm.nih.gov/compound/catechol#section=Safety-and-Hazards

BEFORE STARTING

Use [M]100 Milimolar (mM) catechol water solution as stock, and use [M]10 Milimolar (mM) as working solution.

We use LB cell culture (BL21 strain in our project) to perform measurement.

Add cell culture of a good state (logarithm phase) to 96-well plate.

Manual steps

1 Add 100 µl cell culture per well.

It is recommanded to use replicates and controls to avoid mistakes or deviation.

2 Turn on the microplate reader and computer. Run the software to set the protocol and plate layout.

Protocol for the instrument

3 Shake the plate for 00:00:05 at 600 rpm .

4 Measure the absorbance of cell culture at 600 nm as the estimation of cell amounts.

5 Measure the absorbance of cell culture at 377 nm as the baseline of A377 before the reaction.

6 Plate out and add 2.5 µl catechol ([M]10 Milimolar (mM)) to every well, then plate in immediately.

The working concentration of substrate is [M]0.25 Milimolar (mM) here. Other working concentration could also work.

7 Shake the plate for 00:00:05 at 600 rpm .

8 Measure the absorbance of cell culture at 377 nm, continuously (like 00:00:10 per reading) or after a certain period of time (like 00:01:30).

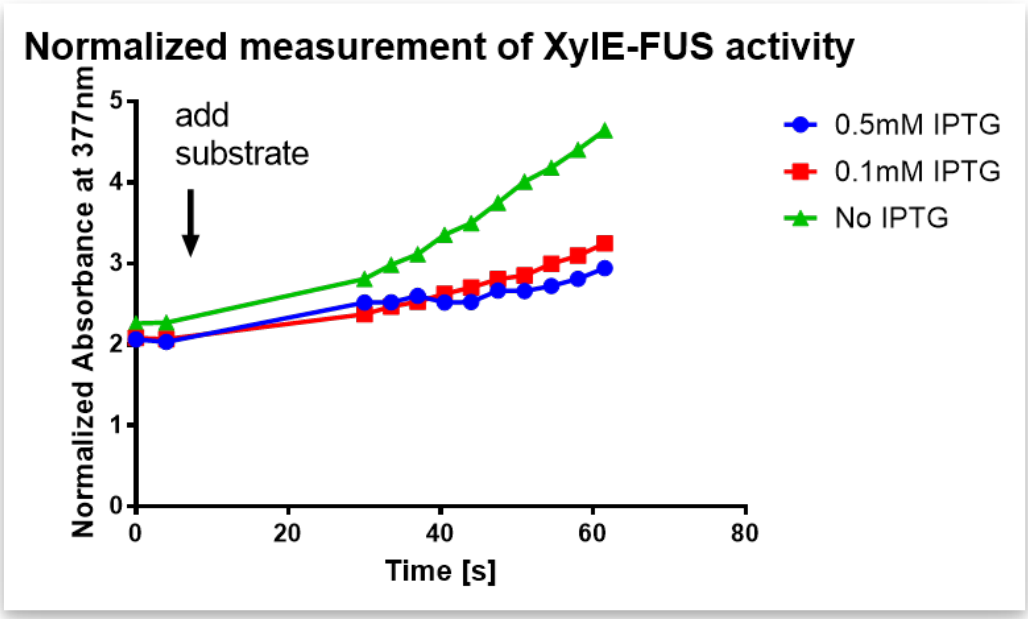
Manual steps

9 Export the data from the software.

Properly dispose of the contaminated cell culture and microplates!

10 Normalize the A337 value by optical density at 600 nm.

Make a Time-A377 plot like this:



Or simply substract the absorbance before the reaction from the value after a specifc period of time: