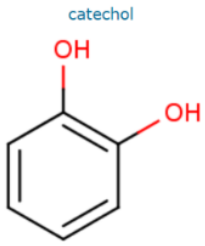
Oct 18, 2019

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



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Ji Gao[1]

[1]Tsinghua University

Ji Gao
Tsinghua University

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

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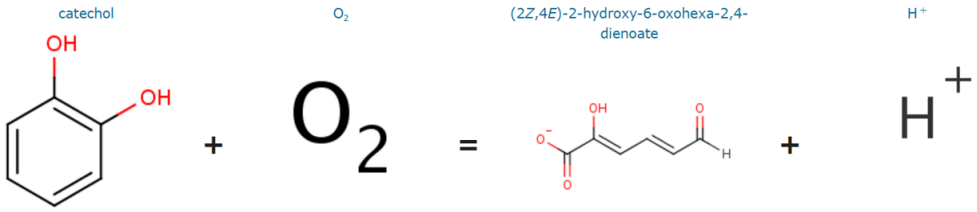
Keywords: microplate reader, enzyme activity by microplate reader, dioxygenase, simple measurement of xyle, measurement of xyle, enzyme activity, dihydroxybenzene, product of the enzyme, enzyme, catechol, photometric mode of microplate reader, xyle

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.

Equipment	
Varioskan LUX Multimode Microplate Reader	NAME
microplate reader	TYPE
Thermo Scientific	BRAND
VL0000D2	SKU
https://www.thermofisher.com/order/catalog/product/VL0000D2?SID=srch-srp-VL0000D2	LINK

Perform the measurement at Room temperature or 25 °C .

Materials

MATERIALS

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

Troubleshooting

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 start

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

> Note
>
> 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 millimolar (mM)) to every well, then plate in immediately.

> Note
>
> The working concentration of substrate is [M] 0.25 millimolar (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.

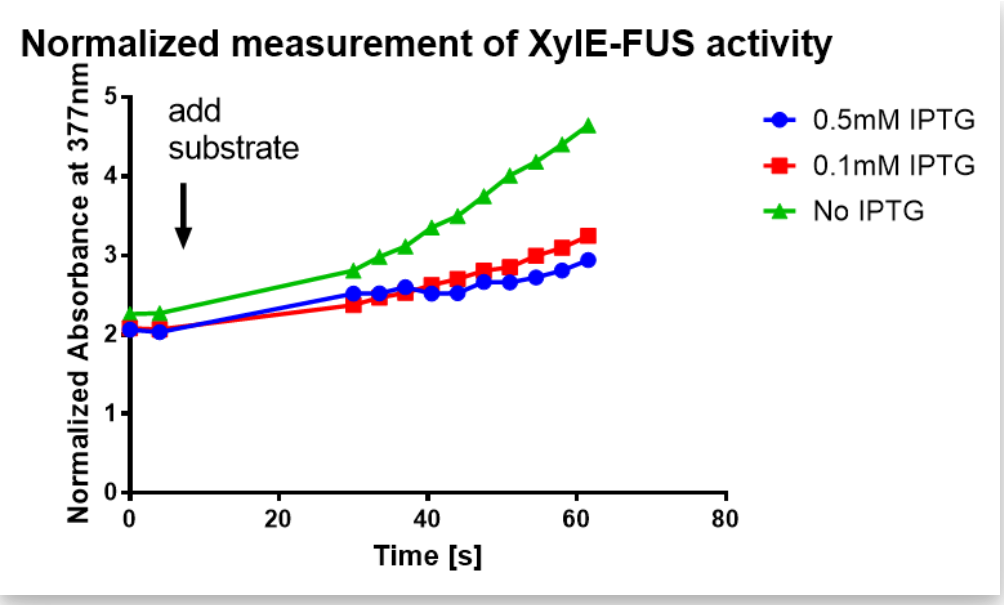
Safety information

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:

Expected result



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

Expected result