

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

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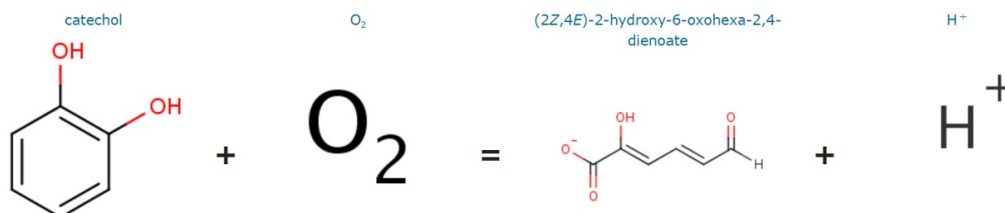
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ABSTRACT

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 μ l cell culture per well.



It is recommended 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 μ l 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

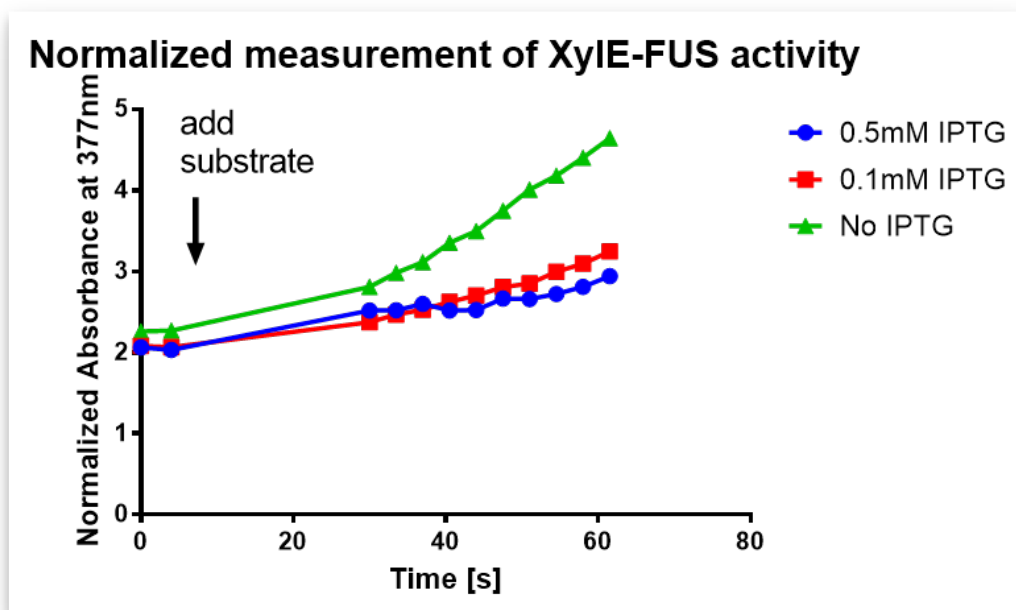
- 9 Export the data from the software.



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 subtract the absorbance before the reaction from the value after a specific period of time:

