AUG 13, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.6gthbwn

Protocol Citation: Tjusls China 2019. 15 Determination of Enzyme Activity.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.6gthbwn>

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

Created: Aug 13, 2019

Last Modified: Aug 13, 2019

PROTOCOL integer ID:
 26867

15 Determination of Enzyme Activity

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MATERIALS

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- ⊗ Ultrasonic Processor **Cole-Parmer Catalog #UX-04714-52**
- ⊗ DMSO **Sigma Catalog #D8418**
- ⊗ General 96-well plates (Black) **Contributed by users Catalog #/**
- ⊗ Infinite M1000 Pro Automatic Microplate Reader **Contributed by users Catalog #/**
- ⊗ Multi-channel adjustable pipette **Contributed by users Catalog #/**
- ⊗ Fluorescent Probe(CDC-1) **Contributed by users Catalog #/**
- ⊗ Target Enzyme(beta-lactamase) **Contributed by users Catalog #/**

System setup - protein concentration

- 1 Soak the 96-well plates in 75% ethanol and put the container in ultrasonic cleaner for 30min to 1 hour, then use ddH₂O to wash these plates several times. Put these clean plates in drying oven at

55°C.

- 2 Dilute the enzyme using its buffer. There we pipet 1 μL protein stock solution in 1mL buffer and mix gently. Then pipet 100 μL protein solution and mix with 400 μL buffer each time, in order to dilute it as a 5-time gradient.
- 3 Pipet 94 μL protein solution into 9 wells in plate, usually choosing B2-D4 area, to set 3 parallel controls. Pipet 94 μL buffer without protein into 3 wells as negative controls. Then add 6 μL fluorescent substrate into wells.
- 4 Set up the program in Infinite M1000 Pro Automatic Microplate Reader.
Shake for 10 sec at 654 rpm.
A digital interface showing a timer set to 00:00:10 and a speed control set to 654 rpm.
Kinetic Cycle (to read fluorescent intensity each cycle)
Fluorescent measure, 75 cycle, 10sec for each cycle.
- 5 Put the plate in Microplate reader, and click Start button.
- 6 When the facility ends testing, save data and import it into GraphPad Prism Software. Use “nonlinear fit” – “straight line” and compare R^2 of lines under different concentrations to pick up the best linear fit one, whose R^2 is most close to 1 .
- 7 Take this concentration as standard value, then set up parallel gradient of its 2x, 0.5x, 0.25x, etc. Repeat step 3-5.
- 8 Calculate the ratio of emission($rE = Q_0/Q_m$, Q_0 means the maximum fluorescent intensity of each reaction under different protein concentrations, Q_m means the maximum fluorescent intensity of all reactions under different protein concentrations). Use GraphPad Prism Software to calculate EC80 value. Set log(concentration of protein) as X, the rate of emission as Y. Use “nonlinear fit” – “log(agonist) vs. response—Find ECanything”, input 80 as the value of F parameter.
- 9 Usually we use the EC80 value as suitable protein concentration, and it can be adjusted according to the actual situation.

System setup - buffer

- 10 Design experimental groups with the “N+(N-1) principle”.
Since we use PBS as our protein buffer, and class B beta-lactamases are depend on Zn^{2+} , so we choose the concentration of NaCl, the concentration of ZnCl_2 , and pH, as variables.
- 11 Repeat step 3-5 to measure.
- 12 When the facility ends testing, save data and import it into GraphPad Prism Software. Use “nonlinear fit” – “straight line” to calculate the initial velocity of each reaction a.k.a. its slope value. Choose the condition with higher initial velocity.

Kinetic Constant Measurement

- 13 Dilute protein again with the ensured most suitable solution into proper concentration.
- 14 Dilute the fluorescent substrate as 2-time gradient for 8 groups.
- 15 Repeat step 3-5 to measure.
- 16 When the facility ends testing, save data and import it into GraphPad Prism Software. Use “nonlinear fit” – “straight line” to calculate the initial velocity of each reaction a.k.a. its slope value.

- 17 Use “nonlinear fit” – “Michaelis-Menten” to fit Michaelis plot of this beta-lactamase. At the same time the software will calculate kinetic constants K_m , V_{max} automatically.
- 18 Dilute protein as 2-time gradient for several groups. Repeat step 3-5 to measure. Take FI as Y, [S] as X, then use “nonlinear fit” – “straight line” to calculate fluorescent calibration value.
- 19 Calculate k_{cat} value. $K_{cat} = V_{max}/[E]$.