

Aug 13, 2019

① 15 Determination of Enzyme Activity

DOI

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

TjusIs China¹

¹Tianjin University



Tjusls China

Tianjin University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account





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

Protocol Citation: TjusIs China 2019. 15 Determination of Enzyme Activity. protocols.io

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

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: August 13, 2019



Last Modified: August 13, 2019

Protocol Integer ID: 26867

Keywords: determination of enzyme activity, enzyme activity

Materials

MATERIALS

- ₩ Ultrasonic Processor Cole-Parmer Catalog #UX-04714-52
- Merck MilliporeSigma (Sigma-Aldrich) Catalog #D8418
- General 96-well plates (Black) Catalog #/
- Mainting Infinite M1000 Pro Automatic Microplate Reader Catalog #/
- Multi-channel adjustable pipette Catalog #/
- X Fluorescent Probe(CDC-1) Catalog #/

Troubleshooting

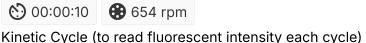


System setup – protein concentration

Soak the 96-well plates in 75% ethanol and put the container in ultrasonic cleaner for 30min to 1 hour, then use ddH_2O to wash these plates several times. Put these clean plates in drying oven at 55°C.



- Dilute the enzyme using its buffer. There we pipet 1 μ L protein stock solution in 1mL buffer and mix gently. Then pipet 100 μ L protein solution and mix with 400 μ L buffer each time, in order to dilute it as a 5-time gradient.
- Pipet 94 μ L protein solution into 9 wells in plate, usually choosing B2-D4 area, to set 3 parallel controls. Pipet 94 μ L buffer without protein into 3 wells as negative controls. Then add 6 μ L fluorescent substrate into wells.
- 4 Set up the program in Infinite M1000 Pro Automatic Microplate Reader. Shake for 10 sec at 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.
- When the facility ends testing, save data and import it into GraphPad Prism Software.

 Use "nonlinear fit" "straight line" and compare R² of lines under different concentrations to pick up the best linear fit one, whose R² is most close to 1.
- Take this concentration as standard value, then set up parallel gradient of its 2x, 0.5x, 0.25x, etc. Repeat step 3-5.
- 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 emissionas 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 Zn²⁺, so we choose the concentration of NaCl, the concentration of ZnCl₂, 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 Km, Vmax 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 kcat value. Kcat = Vmax/[E].