17 Inhibition Kinetics Measurement V.1

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 MATERIALS

DMSO P212121 Catalog #GB-D-360

Scale Contributed by users

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 #/

Ultrasonic Cleaner Contributed by users

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

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**IC50 in vitro test**

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 inhibitors using 100% DMSO to 8mM (a.k.a. 80μM in reaction system) and dilute in 2-time gradients for 12 concentrations. Dilute the enzyme using its buffer to standard concentration (same as the determination of Km).

3. Add the reaction system into 96-well plates. Pipet 94μL protein solution and 1μL inhibitor solution(diluted as 2-time gradients, at least 8 different concentrations), incubated at room temperature for 5 mins, then add substrate CDC-1 5μL.

4. Set controls.  
   - Negative control: 94μL protein solution, 1μL DMSO, 5μL substrate  
   - Blank control: 94μL protein buffer, 1μL DMSO, 5μL substrate  
   And there are 3 parallel holes for each system.

5. 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, 20 cycle, 30sec for each cycle

6. Put the plate in Microplate reader, and click Start button.

7. When the facility ends testing, save data and import the data of 0-200s into GraphPad Prism Software. Use “nonlinear fit” – “straight line” and regulate the number of data to fit $R^2$.

8. Move baseline of blank control. Calculate $Ir = (1-Vr/V0)*100\%$. Take average $Ir$ of each inhibitors’
concentrations as Y value, and take log[1] as X value. Then use “nonlinear fit” – “log(inhibitor) vs. normalized response – Variable slope” to fit IC50 curve, and its value would be calculated automatically.

Judging of inhibition mechanism

9 reversible/irreversible inhibition
Set up gradient concentrations of protein(revolves around the value in screening system) As well as gradient concentrations of inhibitor(revolves around the value of IC50) Draw the plot of $V_0$-[E] to see the movement of straight line. If they are parallel with each other, the mechanism is irreversible. If they are all through the origin, the mechanism is reversible.

10 competitive/noncompetitive inhibition
Set up gradient concentrations of substrate(revolves around the value in screening system) As well as gradient concentrations of inhibitor(revolves around the value of IC50) Draw the plot of $1/V_0-1/[S]$ to see the movement of straight line (Lineweaver-Burk plot). If they gather at Y axis, the mechanism is competitive. If they pass the same point in X axis, the mechanism is noncompetitive. If they gather at another point in this dimension, it would be mixed type competition. (uncompetitive inhibition)

11 After decision of this inhibitor’s mechanism, a constant $K_i$ can be calculated via different equations.[1]
For competitive inhibition,

For noncompetitive inhibition,

For uncompetitive inhibition,

Also, we use a website tool (https://bioinfo-abcc.ncifcrf.gov/IC50_Ki_Converter/index.php) to calculate its value automatically.