Oct 03, 2019

16 High Throughput Screening with Fluorescent Probe

TJUSLS China[1]

[1]Tianjin University

1 Works for me dx.doi.org/10.17504/protocols.io.6guhbww

TJUSLS China
Tianjin University

ABSTRACT

High Throughput Screens (HTS) are recent scientific methods relevant to the field of chemistry and biology, in which hundreds of thousands of experimental samples are subjected to simultaneous testing under given conditions. The sample themselves may take the form of biochemical agents such as chemical compounds, amino acids, or live cells. With the development of laboratory robotics that automate sample preparation, handling and data analysis, scientists can easily and reliably generate and use large datasets from these HTS to answer complex biological questions. HTS is now widely used in the field of pharmaceuticals, biotech and academic institutes for drug discovery, target validation and the identification of genes or proteins that modulate a particular biological pathway.

* Reference
https://www.singerinstruments.com/resource/what-is-high-throughput-screening/

MATERIALS

NAME	CATALOG #	VENDOR
Ultrasonic Processor	UX-04714-52	Cole-Parmer
DMSO	9224	J.T. Baker
General 96-well plates (Black)	/	
Infinite M1000 Pro Automatic Microplate Reader	/	
Multi-channel adjustable pipette	/	
Fluorescent Probe(CDC-1)	/	
Target Enzyme(beta-lactamase)	/	
Buffer(1XPBS with 25μM ZnCl2 pH=8.0)	/	
FDA approved drug library	/	

Sample preparation

1 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 clean plates in drying oven at 55°C.

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 200 μL protein solution then mix with 12.6mL buffer to reach our aiming concentration(1.51nM in reaction system).

3 Dilute the substrate(fluorescent probe CDC-1) with DMSO to reach aiming concentration(10.2μM in reaction system).

Sample handling

4 Pipet 94 µL protein solution into each well of 96-well plates using multi-channel pipette.

5 Pipet 1µL compounds from FDA approved drug library into each well except for the first line of the plate. Pipet 1µL 100% DMSO into wells in the first line as negative controls. Then incubate the protein with compounds at room temperature for 5 min.

6 Pipet 5 µL substrate into each well of 96-well plates using multi-channel pipette quickly.

Readouts and Data Acquisition

7 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, 25 cycle, 30sec for each cycle

8 Put the plate in Microplate reader, click Start button.

9 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.

10 Compare the values of wells which has added compounds with negative control. Calculate the residue activity(Ra = V_r/V_0*100%) and inhibition ratio(Ir = 1- V_r/V_0 *100%). Choose the compounds with Ra<20%, that is to say, Ir>80%, to screen again.

Repeat

11 Repeat step 4-8 to screen again. Only to add chosen compounds and set three same wells as parallel experiments. Collect data and calculate Ir more precisely.

12 Fluorescence quenching experiment: pipet 94µL protein and 5µL fluorescent substrate and mix them. Let it stand still for 30 mins. Then test its maximum fluorescent intensity(Q1). Pipet 1 µL positive compounds then test again(Q2). Calculate the fluorescence quenching rate Qr = (Q1 – Q2)/Q1 *100%.

13 Ascertain the inhibitors, whose Ir is more than 80% while Qr is less than 20%. Then some inhibition kinetic constant can be measured.