（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-D011

Specification：48T/96T

Measuring instrument：Microplate Reader（330-350 nm）

Elabscience® 3-Hydroxy-3-methylglutaryl-CoA Reductase (HMGR) Inhibitor Screening Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Intended use

This kit is used for the determination of the inhibitory effect of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) inhibitors.

Detection principle

3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) is the rate-limiting enzyme of mevalonate pathway, which is the metabolic pathway of acetyl-CoA to produce cholesterol. This detection kit can screen inhibitors of HMGR, which is an important tool for basic research of cholesterol and other related metabolic pathways.

The detection principle of this kit: HMGR catalyzed substrate reaction consumes NADPH, and the absorbance decreases at 340 nm. The addition of inhibitors will inhibit HMGR enzyme activity, resulting in a decrease in the rate of absorbance decline. The inhibition rate can be calculated by measuring the change value of absorbance.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	40 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Enzyme Reagent	0.12 mL × 1 vial	0.24 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	10 mM Lovastatin	0.05 mL × 1 vial	0.05 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Substrate	0.4 mL × 1 vial	0.8 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Accelerant	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months, shading light
	UV Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions

in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (330-350 nm, optimum wavelength: 340 nm), Incubator (25℃)

Reagents:

DMSO

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of enzyme working solution：

Before testing, please prepare sufficient enzyme working solution according to the test wells. For example, prepare 50 μL of enzyme working solution (mix well 5 μL of enzyme reagent and 45 μL of buffer solution). The enzyme working solution should be prepared on spot and put it on ice protected from light. The enzyme working solution should be used up within 1 day.

③ The preparation and use of lovastatin：

Dilute lovastatin to desired concentration with buffer solution. This is HMGR inhibitor, which can as a positive control, measuring inhibition rate can be used as a reference. In this kit, the IC_{50} is about 10 μmol/L, and the measured data may be different.

④ The preparation of substrate working solution：

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 30 μL of substrate working solution (mix well 10 μL of substrate and 20 μL of buffer solution). The substrate working solution should be prepared on spot and put it on ice protected from

light. The substrate working solution should be used up within 1 day.

⑤ The preparation of accelerant working solution:
Dissolve one vial of accelerant with 0.72 mL of double distilled water, mix well to dissolve and put it on ice protected from light during use. Aliquoted storage at -20°C for 3 days protected from light, and avoid repeated freeze/thaw cycles is advised.

⑥ The preparation of reaction working solution:
For each well, prepare 180 μL of reaction working solution (mix well 140 μL of buffer solution, 20 μL of substrate working solution and 20 μL of accelerant working solution). The reaction working solution should be kept at room temperature protected from light for at least 5 min before use. The reaction working solution should be used up within 1 day.

Sample preparation

It is recommended to use buffer solution as a sample solvent for compounds. If the sample is poorly water-soluble, DMSO can be prepared into a high-concentration solution and then diluted with buffer solution. The DMSO content in the reaction system should be less than 5%.

Operating steps

① Blank well: Add 40 μL of buffer solution to the wells;
Total enzyme well: Add 20 μL of buffer solution and 20 μL of enzyme working solution to the wells;
Positive control well: Add 20 μL of enzyme working solution and 20 μL of lovastatin to the wells;
Sample well: Add 20 μL of enzyme working solution and 20 μL of sample to the wells.

② Add 180 μL of reaction working solution into each well.

③ Mix fully with microplate reader for 5 s. Measure the OD values of each well at 340 nm with microplate reader, as A_1. Incubate at 25℃ for 20 min, measure the OD values of each well at 340 nm with microplate reader, as A_2.

(The positive control well is the inhibition rate of lovastatin, a specific inhibitor of HMGR, which can only be used as a reference. This well is not needed in the actual determination process. In this kit, the IC_{50} is about 10 μmol/L, and the measured data may be different.)

Calculation

$$\text{Inhibition rate (\%)} = (\Delta A_1 - \Delta A_2) \div \Delta A_1 \times 100\%$$

[Note]

$\Delta A_1 = \Delta A_{\text{total}} - \Delta A_{\text{blank}}$.

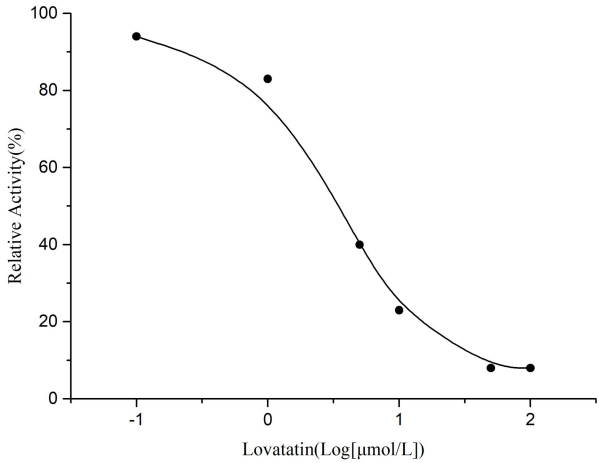
$\Delta A_2 = \Delta A_{\text{sample}} - \Delta A_{\text{blank}}$.

$\Delta A = A_1 - A_2$.

Appendix I Performance Characteristics

Inhibition curve

The effect of HMGR inhibitor screening kit for detection of lovastatin inhibitor.



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.