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

Catalog No：E-BC-D008

Specification：48T/96T

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Elabscience® Ferroptosis Suppressor Protein-1 (FSP-1) 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
Email: techsupport@elabscience.com
Website: www.elabscience.com

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 ferroptosis suppressor protein-1 (FSP-1) inhibitors.

Detection principle

Ferroptosis suppressor protein-1 (FSP-1) induces non-Caspase-dependent apoptosis based on amino acid series C-terminal fragment, nuclear translocation, overexpression and other factors. The FSP-1-CoQ10-NAD(P)H pathway, parallel to the classical glutathione (GSH)-GPX4 pathway, can protect cells from ferroptosis, and play a "mixed blessing" role in cell life activities.

The detection principle of this kit: FSP-1 catalyzes the substrate reaction to produce fluorescence products, and the inhibitor will inhibit the activity of FSP-1 and reduce the rate of the rise of fluorescence value. The inhibition rate can be calculated by measuring the relative change value of its enzyme activity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	14 mL × 1 vial	28 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Substrate	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months, shading light
Reagent 3	Enzyme Diluent	0.6 mL × 1 vial	1.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Enzyme Agent	0.15 mL × 1 vial	0.3 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Inhibitor	0.1 mL × 1 vial	0.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	Chromogenic Agent	0.1 mL × 1 vial	0.2 mL × 1 vial	-20°C, 12 months, shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator (37℃)

Reagent preparation

① Keep enzyme agent on ice before use to thawing. Equilibrate other reagents to 25℃ before use.

② The preparation of substrate working solution：
Dissolve one vial of substrate with 0.6 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 7 days protected from light.

③ The preparation of 1 mmol/L inhibitor working solution：
Before testing, please prepare sufficient 1 mmol/L inhibitor working solution according to the test wells. For example, prepare 100 μL of 1 mmol/L inhibitor working solution (mix well 5 μL of inhibitor and 95 μL of buffer solution). The 1 mmol/L inhibitor working solution should be prepared on spot and keep it on ice protected from light. Use preparaed solution within 1 day. (This reagent is an FSP-1 inhibitor, as a positive control, the determination of inhibition rate can be used as a reference, IC_{50} is about 10 μmol/L).

④ The preparation of chromogenic stock solution：
Before testing, please prepare sufficient chromogenic stock solution. For example, prepare 100 μL of chromogenic stock solution (mix well 5 μL of chromogenic agent and 95 μL of buffer solution). Aliquoted storage at -20°C for 10 days protected from light.

⑤ The preparation of chromogenic working solution：
For each well, prepare 60 μL of chromogenic working solution (mix well 5 μL of chromogenic stock solution and 55 μL of buffer solution). Aliquoted storage at -20°C for 10 days protected from light. The chromogenic working solution should be prepared on spot protected from light and used up within 1 day.

⑥ The preparation of enzyme working solution：
Before testing, please prepare sufficient enzyme working solution. For

example, prepare 40 μL of enzyme working solution (mix well 10 μL of enzyme agent and 30 μL of buffer solution). The enzyme working solution should be prepared on spot and keep it on ice protected from light. Use preparaed solution within 5 h.

The key points of the assay

It should be ensured that the sample is fully mixed with the enzyme working solution.

Operating steps

① Blank well: Add 10 μL of enzyme diluent to the wells;
Sample well: Add 10 μL of enzyme working solution to the wells.
Total enzyme well: Add 10 μL of enzyme working solution to the wells;
Positive control well: Add 10 μL of enzyme working solution to the wells;

② Add 15 μL of buffer solution into blank wells and total enzyme wells. Add 15 μL of samples into sample wells. Add 15 μL of 1 mmol/L inhibitor working solution into positive control wells.

③ Incubate at 37℃ for 10 min protected from light.

④ Add 15 μL of substrate working solution into each well.

⑤ Add 60 μL of chromogenic working solution into each well.

③ Mix fully with fluorescence microplate for 5 s and incubate at 25℃ for 10 min protected from light. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

$$\text{Inhibition rate (\%)} = (F_1 - F_2) \div (F_1 - F_3) \times 100\%$$

[Note]

F_1: The fluorescence value of total enzyme well.

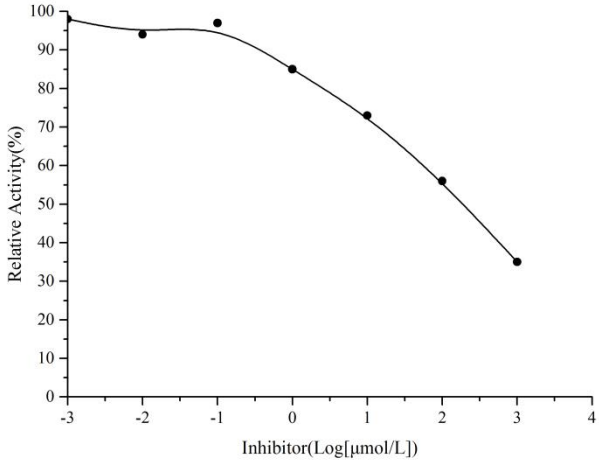
F_2: The fluorescence value of sample well.

F_3: The fluorescence value of blank well.

Appendix I Performance Characteristics

Inhibition curve

The effect of FSP-1 inhibitor screening kit for detection of FSP-1 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.