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

Catalog No：E-BC-F022

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.15-5.00 U/L

Elabscience® Ferroptosis Suppressor Protein-1 (FSP-1) Activity Fluorometric Assay 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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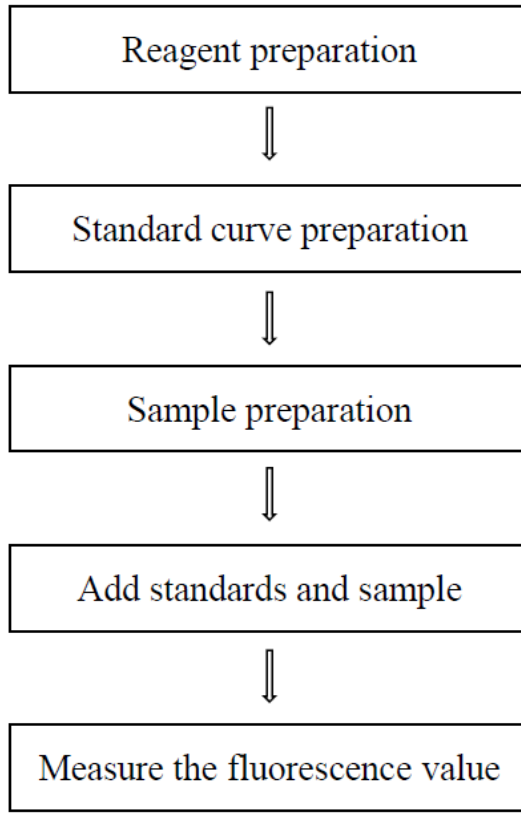
Assay summary



Intended use

This kit can be used to measure ferroptosis suppressor protein-1 (FSP-1) activity in cell, animal and plant tissue samples.

Detection principle

Ferroptosis suppressor protein-1(FSP-1) induce non-caspase-dependent apoptosis based on factors such as C-terminal fragment of amino acid series, nuclear translocation and overexpression. Through the FSP1-CoQ10-NAD(P)H pathway and the classical glutathione (GSH)-GPX4 pathway, FSP-1 can protect cells from ferroptosis, and plays a "double-edged sword" role in cell life activities.

The detection principle of this kit: FSP-1 catalyzes the substrate reaction to generate NADH, so that its fluorescence value increases under the excitation wavelength of 535 nm and emission wavelength of 587 nm. Adding inhibitors will inhibit the activity of FSP-1 and cause the rate of increase in fluorescence value to decrease, and the activity of FSP-1 can be calculated by measuring the difference value.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	40 mL ×2 vials	-20 ℃, 12 months shading light
Reagent 2	Substrate	Powder ×2 vials	-20 ℃, 12 months shading light
Reagent 3	Inhibitor	0.08 mL ×1 vial	-20 ℃, 12 months shading light
Reagent 4	Chromogenic Agent	0.05 mL ×1 vial	-20 ℃, 12 months shading light
Reagent 5	1 mmol/L Standard Solution	1 mL ×1 vial	-20 ℃, 12 months shading light
	Black 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:

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

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to 25℃ before use. Please centrifuge the chromogenic agent before use.

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

③ The preparation of inhibitor working solution:
Before testing, please prepare sufficient inhibitor working solution according to the test wells. For example, prepare 100 μL of inhibitor working solution (mix well 5 μL of inhibitor and 95 μL of buffer solution). Aliquoted storage at -20 ℃ for 3 days.

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 ℃ for 10 days.

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). The chromogenic

working solution should be prepared on spot protected from light and used up within the same day.

⑥ The preparation of 100 μmol/L standard solution:

Before testing, please prepare sufficient 100 μmol/L standard solution. For example, prepare 1000 μL of 100 μmol/L standard solution (mix well 100 μL of 1 mmol/L standard solution and 900 μL of buffer solution). The 100 μmol/L standard solution should be prepared on spot.

⑦ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 20, 30, 40, 50, 60, 70, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**20**	**30**	**40**	**50**	**60**	**70**	**100**
100 μmol/L Standard (μL)	0	40	60	80	100	120	140	200
Buffer solution (μL)	200	160	140	120	100	80	60	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL buffer solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared sample within 4 h.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Homogenize 1×10^6 cells in 200 μL buffer solution with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared sample within 4 h.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	2-5
10% Mouse heart tissue homogenate	1
10% Mouse kidney tissue homogenate	1-2
10% Mouse lung tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Green beans tissue homogenate	1

1×10^6 HL-60 cells	1
1×10^6 Hela cells	1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The inhibitor working solution and chromogenic stock solution should be mixed well before use.

② The standard solution should be prepared on spot to avoid being oxidized.

③ After adding inhibitor working solution to the sample wells, it is necessary to ensure that the sample and inhibitor working solution are mixed well.

Operating steps

① Standard well: add 10 μL of standard with different concentrations into the well.

Sample well: add 10 μL of sample into the well.

Control well: add 10 μL of sample into the well.

② Add 15 μL of buffer solution into standard and control wells.

Add 15 μL of inhibitor working solution into sample wells.

③ Incubate at 37 ℃ for 20 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.

⑥ Mix fully with fluorescence microplate reader for 5s. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm for sample and control wells, as F_1.

⑦ Incubate at 37 ℃ for 20 min protected from light. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm for each well, as F_2. (The standard wells only need to measure F_2.)

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean F_2 value of the blank (Standard #①) from all standard readings. This is the absoluted F_2 value.

3. Plot the standard curve by using absoluted F_2 value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Tissue sample:

Definition: The amount of enzyme in 1 kg tissue per 1 min that product 1 μmol resorufin at 25 ℃ is defined as 1 unit.

$$\text{FSP-1 activity (U/Kg wet weight)} = (\Delta F_{control} - \Delta F_{sample} - b) \div a \times f \div \frac{m}{V} \div T$$

2. Cell sample:

Definition: The amount of enzyme in 1×10^9 cells per 1 min that product 1 μmol resorufin at 25 ℃ is defined as 1 unit.

$$\text{FSP-1 activity (U/10^9)} = (\Delta F_{control} - \Delta F_{sampe} - b) \div a \times f \div \frac{n}{V} \div T$$

[Note]

ΔF_{sample}: $\Delta F = F_2 - F_1$.

$\Delta F_{control}$: $\Delta F = F_2 - F_1$.

f: Dilution factor of sample before tested.

m: The wet weight of tissue, g.

V: The volume of buffer solution in the preparation of sample, mL.

n: The number of cell sample/10^6.

T: Reaction time, 20 min.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver tissue were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.30	4.10	1.60
%CV	5.4	3.0	3.2

Inter-assay Precision

Three mouse liver tissue were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.30	4.10	1.60
%CV	8.0	6.5	7.2

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc.(U/L)	1.3	4.1	1.6
Observed Conc.(U/L)	1.274	4.1	1.568
Recovery rate (%)	98	100	98

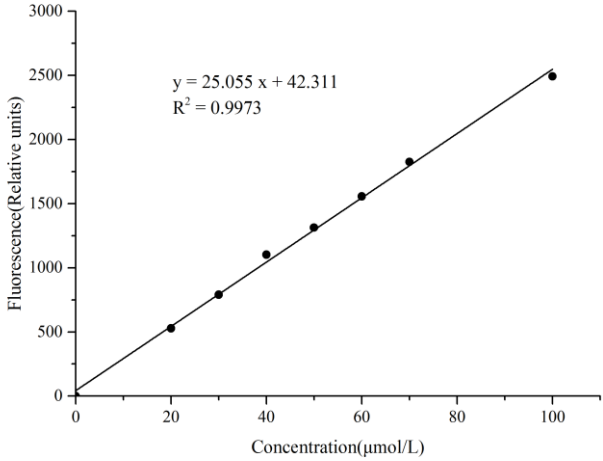
Sensitivity

The analytical sensitivity of the assay is 0.15 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**20**	**30**	**40**	**50**	**60**	**70**	**100**
F_2 value	223	758	1016	1344	1537	1796	2098	2746
	224	744	1013	1306	1537	1764	2000	2683
Average F_2 value	223	751	1013	1325	1537	1780	2049	2715
Absoluted F_2 value	0	528	790	1102	1314	1557	1826	2492



Appendix II Example Analysis

Example analysis:

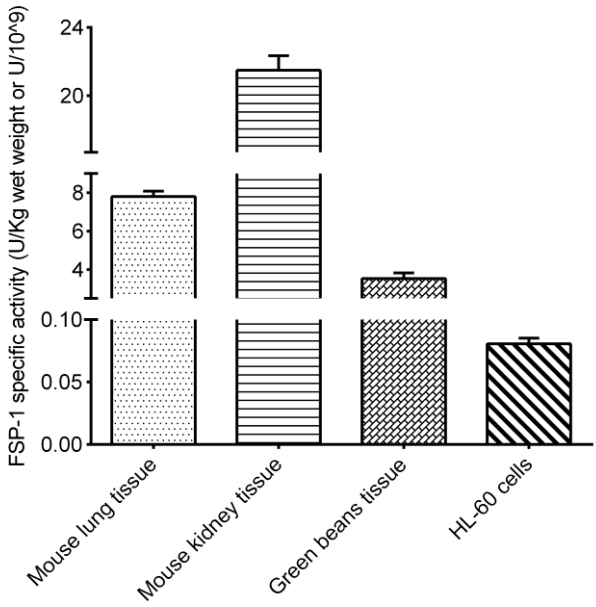
Take 10 μL of 1×10^6 HL-60 cells supernatant and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 25.055 x + 42.311, the average F_1 value of the sample well is 316, the average F_2 value of the sample well is 631, $\Delta F_{sample} = F_2 – F_1 = 631 - 316 = 315$. the average F_1 value of the control well is 329, the average F_2 value of the control well is 899, $\Delta F_{control} = F_2 – F_1 = 899 - 329 = 570$, and the calculation result is:

$$\text{FSP-1 activity (U/10^9)} = (570 – 315 – 42.311) \div 25.055 \div 1 \div 0.2 \div 20$$

$$= 0.085 \text{ U/10^9}$$

Detect 10% mouse lung tissue homogenate, 10% mouse kidney tissue homogenate, 10% green beans tissue homogenate and 1×10^6 HL-60 cells according to the protocol, the result is as follows:



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.