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

Catalog No：E-BC-F066

Specification：96T

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=485 nm/535 nm)

Elabscience® Cystine Uptake 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
Tel: 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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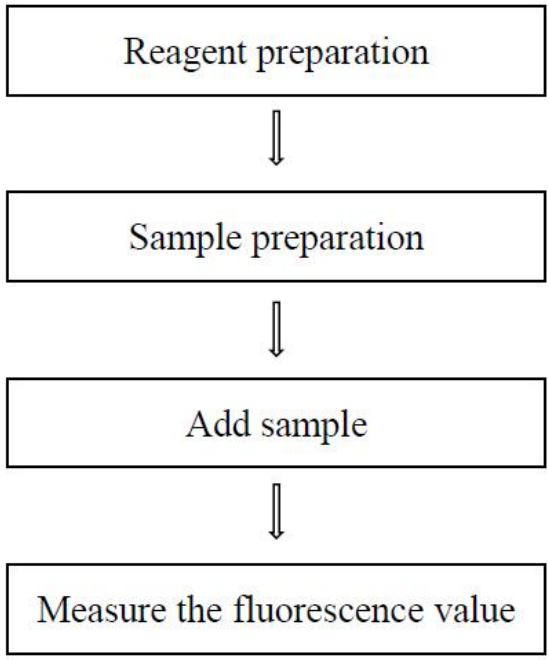
Assay summary



Intended use

This kit can be used to measure cystine uptake ability in cell samples.

Detection principle

Cystine is the source of the antioxidant glutathione, which plays an important role in the redox equilibrium within cells. The cystine/glutamate transporter (xCT) on the cell membrane is one of the amino acid transporters that transports extracellular cystine into the cell and intracellular glutamate into the cell in a ratio of 1:1. xCT regulates intracellular glutathione synthesis through cystine uptake and maintains cellular redox equilibrium. When the xCT activity on the cell membrane decreases, the ability of the cell to uptake cysteine may decrease, which may lead to cell ferroptosis. In recent years, the association between xCT and related diseases such as cancer, neurodegenerative diseases, and immunity has gradually become one of the research hotspots.

This kit is a convenient kit for detecting cystine uptake ability of cells by fluorescence method. Cystine analog, like cystine, can be transported into cells through xCT on the cell membrane. Cystine analog react with fluorescent probes and emit fluorescence. Therefore, the ability of cell cystine uptake can be determined by detecting the fluorescence intensity generated by cystine analog.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer	28 mL × 1 vial	-20℃, 12 months
Reagent 2	Cystine Analog	0.1 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Fluorescence Probe	0.1 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	Reducing Reagent	0.3 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:

Micropipettor, Incubator, Centrifuge, Fluorescence microplate reader (Ex/Em=485 nm/535 nm).

Reagents:

PBS(0.01 M，pH 7.4) (t can be replaced with a serum-free medium without cysteine), Anhydrous ethanol

Reagent preparation

① Equilibrate all the reagents to room temperature（25℃）before use. Aliquot cystine analog storage at -20°C, and avoid repeated freeze/thaw cycles is advised.

② The preparation of cystine analog working solution:
Before testing, please prepare sufficient cystine analog working solution according to the test wells. For example, prepare 500 μL of cystine analog working solution (mix well 1 μL of cystine analog and 499 μL of PBS).The cystine analog working solution should be prepared on spot. Preserve it on ice with shading light for use and it should be used up within 2 h.

③ The preparation of measuring working solution:
Before testing, please prepare sufficient measuring working solution according to the test wells. For example, prepare 1000 μL of cystine analog working solution (mix well 1979 μL of buffer, 1 μL of fluorescence probe and 20 μL of reducing reagent). The measuring working solution should be prepared on spot. Preserve it on ice with shading light for use and it should be used up within 2 h.

The key points of the assay

① If using PBS to wash cells, make sure to prepare a sufficient amount before the experiment.

② Cystine analog working solution should be pre-heated in a 37℃ incubator for 2-5 minutes and the prepared solution should be used up within 2 h.

Operating steps

Suspension cells

① Take 2 mL centrifuge tubes as an example, the number of cells per tube should be at least 1×10^5, washed twice with PBS, centrifuged at room temperature, 500×g for 3 min, and it is recommended to set a negative cell control for each experiment.

② Resuspend the negative control cell group with 200 μL of PBS, and resuspend the sample cell group with 200 μL of cystine analog working solution. Incubate at 37°C for 30 min protect from light, this step is the process of cell uptake of cystine analog. Due to differences in cell types and numbers, the incubation time needs to be explored by pre-experiments.

③ Wash cells with PBS twice, centrifuge at room temperature at 500×g for 3 min, and discard the supernatant. The supernatant was absorbed completely as far as possible, otherwise it would affect the subsequent chromogenic reaction.

④ Add 200 μL of absolute ethyl alcohol to the negative control cell group and sample cell group to lyse the cells.

⑤ Take 50 μL of cell lysate to the black microplate， add 200 μL of measuring working solution to each well, incubate at 37°C for 30 min protect from light.

⑥ Set the excitation wavelength of 485 nm and emission wavelength of 535 nm. Detected by fluorescent microplate reader, the fluorescence value reflects the ability of cells to take up cystine.

Adherent cells:

① Take 96-well plate as an example, the number of cells per tube should be at least 5×10^4, washed twice with PBS, and it is recommended to set a negative cell control for each experiment.

② Add 200 μL of PBS to the negative control cell group, add 200 μL of cystine analog working solution to the sample cell group. Incubate at 37°C for 30 min protect from light, this step is the process of cell uptake of cystine analog. Due to differences in cell types and numbers, the incubation time needs to be explored by pre-experiments.

③ Wash cells with PBS twice and discard the supernatant. The supernatant was absorbed completely as far as possible, otherwise it would affect the subsequent chromogenic reaction.

④ Add 200 μL of absolute ethyl alcohol to the negative control cell group and sample cell group and suck the cells repeatedly by pipette to lyse.

⑤ Take 50 μL of cell lysate to the black microplate，add 200 μL of measuring working solution to each well, incubate at 37°C for 30 min protect from light.

⑥ Set the excitation wavelength of 485 nm and emission wavelength of 535 nm. Detected by fluorescent microplate reader, the fluorescence value reflects the ability of cells to take up cystine.

Example analysis:

293T cells were cultured in 24-well cell culture plates. In the drug group, the ferro ptosis inducer erastin was added with the final concentration of 0.5 μmol/L. After collecting the cells, the results were as follows:

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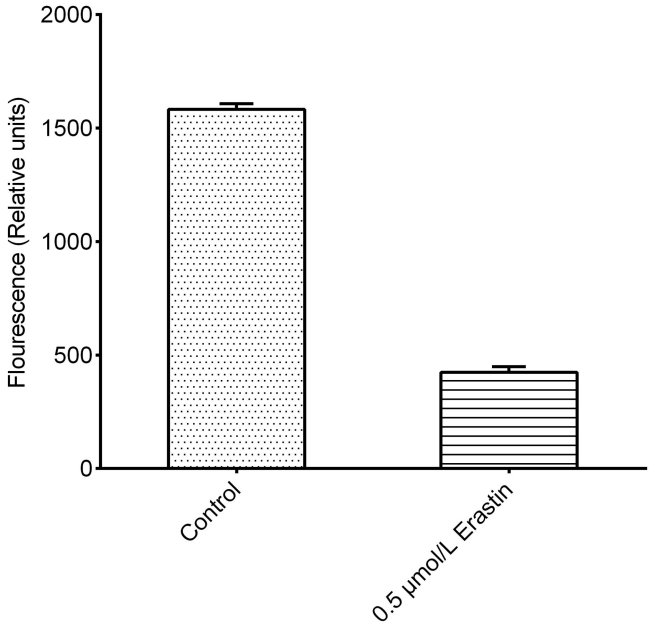

Statement

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.