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

Catalog No：E-BC-F102

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=395 nm/480 nm)

Elabscience® Cellular Cuprous 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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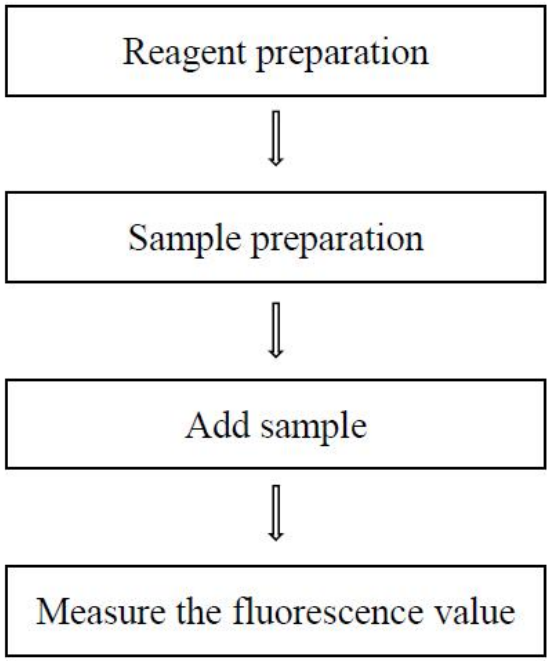
Assay summary



Intended use

This kit can be used to measure cellular cuprous in cell samples.

Detection principle

Abnormal accumulation of cellular cuprous in cells induces the oligomerization of key components of the lipoic acid-modified pyruvate dehydrogenase complex, which affects the tricarboxylic acid cycle, triggers proteotoxic stress, and induces cell death.

Cellular cuprous can catalyze the substrate to produce fluorescent substances, and the higher the copper ion concentration, the more fluorescent substances are generated per unit time. The fluorescence delete can be detected at the excitation wavelength of 395 nm and emission wavelength of 480 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Substrate A	0.8 mL × 1 vial	1.6 mL × 1 vial	-20℃, 12 months, shading light
Reagent 2	Substrate B	6 mL × 1 vial	12 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= 395 nm/480 nm)

Reagents:

Normal saline (0.9% NaCl), Double distilled water

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of substrate A working solution：

For each well, prepare 100 μL of substrate A working solution (mix well 10 μL of substrate A and 90 μL of double distilled water). Store at -20°C for 7 days protected from light.

Sample preparation

① Sample preparation

Cells:

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

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
1×10^6 Hela cells	1
1×10^6 Jurkat cells	1
1×10^6 K562 cells	1
1×10^6 Molt-4 cells	1
1×10^6 293T cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Sample well: add 20 μL of sample into the sample wells;

② Add 100 μL of substrate A working solution into each well.

③ Add 100 μL of substrate B into each well.

④ Mix fully with microplate reader for 5 s and incubate at 37℃ for 10 min protected from light. Measure the fluorescence intensity of each well at the excitation wavelength of 395 nm and the emission wavelength of 480 nm.

Appendix I Example Analysis

Example analysis：

Take 20 μL of cell homogenate supernatant and carry the assay according to the operation steps.The results are as follows:

Sample	fluorescence value
1×10^6 Hela cells	714
1×10^6 Jurkat cells	701
1×10^6 K562 cells	577
1×10^6 Molt-4 cells	474
1×10^6 293T cells	1230

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.