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

Catalog No：E-BC-F059

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=355 nm/460 nm)

Detection range：0.009-0.351 U/L

Elabscience® Cathepsin B 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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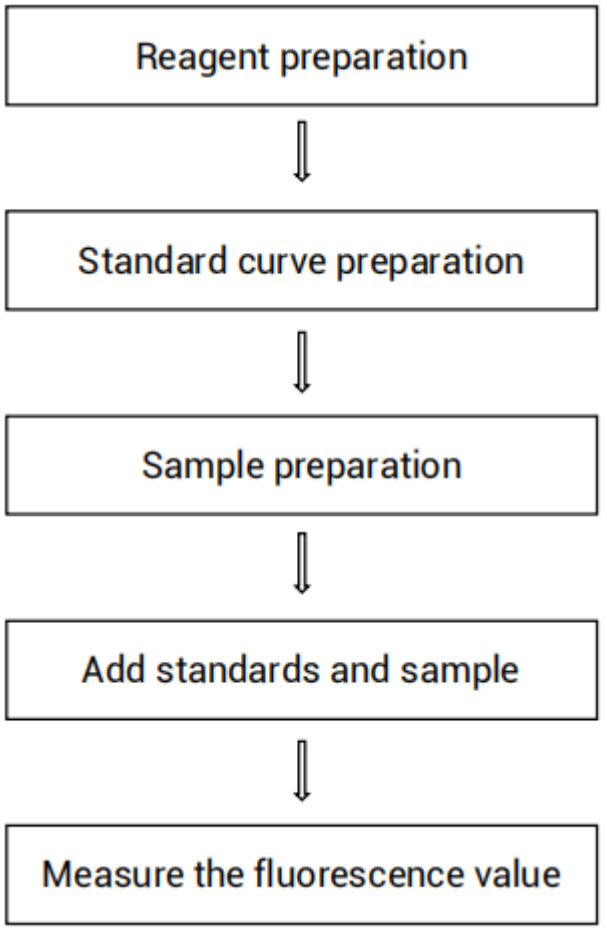
Assay summary



Intended use

This kit can be used to measure cathepsin B activity in animal tissue, plant tissue and cell samples.

Detection principle

Cathepsin B is a cysteine proteolytic enzyme in lysosomes. It is responsible for the hydrolysis, degradation and processing of proteins in organisms, thus maintaining a precise balance between protein synthesis and degradation. The abnormal proteolytic activity of cathepsin and the alteration of its expression pattern are involved in the key steps of the tumor disease process and can be used as markers for certain cancer types, serving as effective regulatory factors for cancer occurrence. As an important type of biomarker, the research on cathepsin is crucial for the study of drug targets and drug development.

The detection principle of this kit: Cathepsin B specifically cleaves the fluorescent substrate, and the fluorescent group of the product emits fluorescence. Fluorescence is detected at an excitation wavelength of 355 nm and an emission wavelength of 460 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	40 mL × 1 vial	40 mL × 2 vials	-20°C, 12 months shading light
Reagent 2	Buffer Solution	10 mL × 1 vial	20 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Substrate	0.025 mL × 1 vial	0.05 mL ×1 vial	-20°C, 12 months shading light
Reagent 4	1 mmol/L Standard Solution	0.2 mL×1 vial	0.4 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=355 nm/460 nm), Incubator

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of substrate working solution:

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 500 μL of substrate working solution (mix well 5 μL of substrate and 495 μL of buffer solution). Keep it on ice during use. Store at -20°C for 2 days protected from light.

③ The preparation of 10 μmol/L standard solution:

Before testing, please prepare sufficient 10 μmol/L standard solution. For example, prepare 1000 μL of 10 μmol/L standard solution (mix well 10 μL of 1 mmol/L standard solution and 990 μL of buffer solution). The 10 μmol/L standard solution should be prepared on spot protected from light and used up within 8 h.

④ The preparation of standard curve:

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

Dilute 10 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 3, 4, 5, 8, 10 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**1**	**2**	**3**	**4**	**5**	**8**	**10**
10 μmol/L Standard (μL)	0	20	40	60	80	100	160	200
Buffer Solution (μL)	200	180	160	140	120	100	40	0

Sample preparation

① Sample preparation

Tissue sample:

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

② Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used up within 4 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell samples:

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

② Lyse 1× 10^6 cells with 200 μL extraction solution. Place on the ice box and mix well every 5 min, lyse for 10 min.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used up within 4 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

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	3-10
10% Mouse kidney tissue homogenate	2-3
10% Mouse muscle tissue homogenate	1
10% Mouse spleen tissue homogenate	3-10
10% Mouse lung tissue homogenate	1
10% Potato tissue homogenate	1
10% Corn tissue homogenate	1
1×10^6 HL-60 cells	1
1×10^6 RAW 264.7 cells	1

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

Operating steps

① Standard well: add 20 μL of standard solution with different concentrations into the wells.
Sample well: add 20 μL of sample into the wells.

② Add 130 μL of buffer solution into standard wells. Add 100 μL of buffer solution into sample wells.

③ Add 30 μL of substrate working solution into sample wells.

④ Mix fully with fluorescence microplate for 5s. Measure the fluorescence at the excitation wavelength of 355 nm and the emission wavelength of 460 nm, as F_1. Incubate at 37°C for 10 min protected from light.Measure the fluorescence at the excitation wavelength of 355 nm and the emission wavelength of 460 nm, as F_2. The standard curve is fitted to the standard well in F_2 value.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.
3. Plot the standard curve by using absoluted fluorescence 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. Animal tissue or cell samples:

Definition: The amount of enzyme in 1 g tissue or cell protein per 1 min that produce 1 μmol of production at 37°C is defined as 1 unit.

$$\text{Cathepsin B activity (U/gprot)} = (\Delta F - b) \div a \div T \times f \div C_{pr}$$

2. Plant tissue sample:

Definition: The amount of enzyme in 1 kg tissue per 1 min that produce 1 μmol of production at 37°C is defined as 1 unit.

$$\text{Cathepsin B activity (U/kg wet weight)} = (\Delta F - b) \div a \div T \times f \times V \div m$$

[Note]

ΔF: $\Delta F = F_2 - F_1$ (The absolute fluorescence value of sample well).

T: Reaction time, 10 min.

f: Dilution factor of sample before tested.

C_{pr}: The concentration of protein in sample, gprot/L.

m: The weight of tissue, kg.

V: The volume of extraction solution, L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse lung 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)	0.10	0.20	0.30
%CV	2.1	4.9	3.6

Inter-assay Precision

Three mouse lung 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)	0.10	0.20	0.30
%CV	5.1	5.3	9.9

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 97%.

	Sample 1	Sample 2	Sample 3
Expected Conc(U/L)	0.10	0.20	0.30
Observed Conc(U/L)	0.094	0.192	0.303
Recovery rate (%)	94.0	96.0	101.0

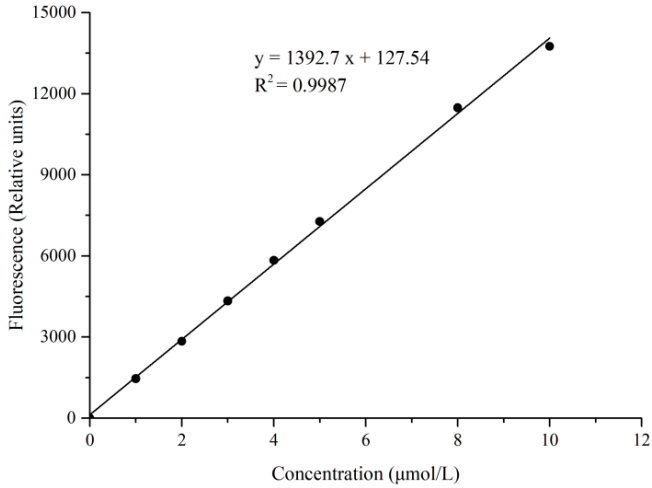
Sensitivity

The analytical sensitivity of the assay is 0.009 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	1	2	3	4	5	8	10
F_2 value	17	1393	2834	4426	5913	7270	11523	14204
	20	1563	2890	4281	5797	7316	11475	13331
Average F_2 value	19	1478	2862	4354	5855	7293	11499	13768
Absoluted F_2 value	0	1460	2844	4335	5837	7275	11481	13749



Appendix Π Example Analysis

Example analysis：

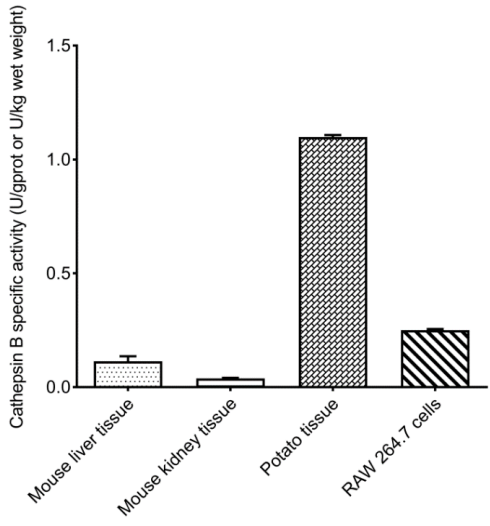
Take 20 μL of 10% mouse liver tissue homogenate which dilute for 10 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 1392.7 x + 127.54, the F_1 value of the sample well is 1268, the F_2 value of the sample well is 4021, $\Delta F = F_2 - F_1$ = 4021 - 1268 = 2753, the concentration of protein is 16.57 gprot/L. The calculation result is:

$$\text{Cathepsin B activity (U/gprot)} = (2753 - 127.54) \div 1392.7 \div 10 \times 10 \div 16.57$$

$$= 0.114 \text{ U/gprot}$$

Detect 10% mouse liver tissue homogenate (the concentration of protein is 16.57 gprot/L, dilute for 10 times), 10% mouse kidney tissue homogenate (the concentration of protein is 12.33 gprot/L, dilute for 2 times), 10% potato tissue homogenate and 1×10^6 RAW 264.7 cells (the concentration of protein is 0.66 gprot/L) 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.