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

Catalog No：E-BC-F052

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

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=328 nm/393 nm)

Detection range：0.02-30.00 U/L

Elabscience® Cathepsin D (CatD) 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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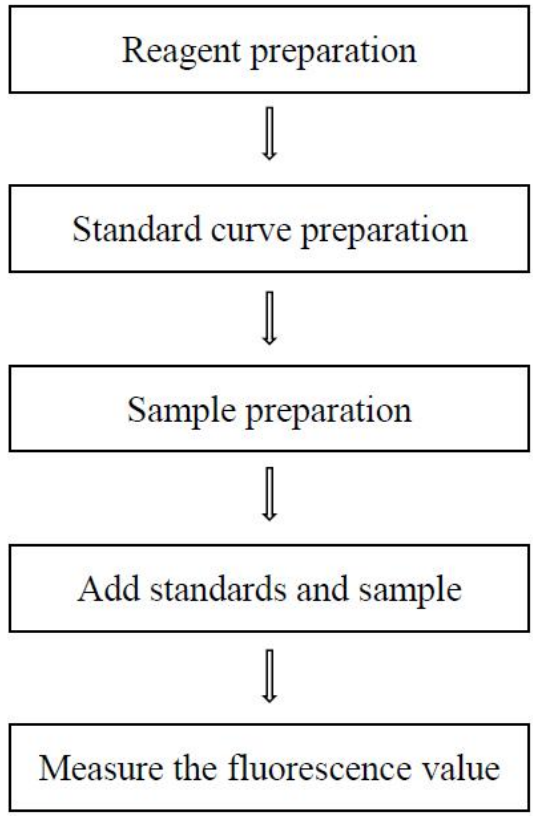
Assay summary



Intended use

This kit can be used to measure cathepsin D (CatD) activity in serum, plasma, animal tissue and cell samples.

Detection principle

Cathepsin D (CatD) is an important intracellular aspartic acid protease and is widely present in the biological world. Cathepsin D has multiple biological functions and is involved in intracellular protein degradation, apoptosis and autophagy, etc.

The detection principle of this kit: CatD hydrolyzes the substrate to generate the chromogenic substance. The activity of cathepsin D in the sample is calculated by measuring the changes in fluorescence values at the excitation wavelength of 328 nm and the emission wavelength of 393 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
Reagent 2	Buffer Solution	11 mL × 1 vial	22 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Substrate	1.1 mL × 1 vial	2.2 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	10 mmol/L Standard Solution	0.1 mL × 1 vial	0.1 mL × 1 vial	-20°C, 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=328 nm/393 nm), Incubator(37°C)

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of working solution:

Before testing, please prepare sufficient working solution according to test wells. For example, prepare 25 μL of working solution (mix well 20 μL of substrate and 5 μL of buffer solution). The working solution should be prepared on spot protected from light and used up within 4 h.

③ The preparation of 300 μmol/L standard solution:

Before testing, please prepare sufficient 300 μmol/L standard solution. For example, prepare 1000 μL of 300 μmol/L standard solution (mix well 30 μL of 10 mmol/L standard solution and 970 μL of buffer solution). Store at 2-8°C for 1 month.

④ The preparation of standard curve:

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

Dilute 300 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 60, 90, 120, 150, 180, 210, 300 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**60**	**90**	**120**	**150**	**180**	**210**	**300**
300 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

Serum or plasma samples: detect directly.

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 extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 12000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared tissue supernatant on the same day.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

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 extraction solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 12000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared cell supernatant on the same day.

⑤ 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
Mouse serum	1-2
Mouse plasma	1
10% Rat pancreas tissue homogenate	1-2
10% Rat spleen tissue homogenate	1
10% Rat small intestine tissue homogenate	1-2
10% Rat brain tissue homogenate	1
10% Rat stomach tissue homogenate	1
10% Rat kidney tissue homogenate	2-4
10% Rat liver tissue homogenate	2-8
1×10^6 Hela cells	1
1×10^6 HL-60 cells	1
1×10^6 293T 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 with different concentrations into the well.

Sample well: add 20 μL of sample into the well.

② Add 20 μL of working solution into each well.

③ Add 160 μL of buffer solution into each well.

④ Mix fully with fluorescence microplate for 5 s. Measure the fluorescence intensity of sample wells at the excitation wavelength of 328 nm and the emission wavelength of 393 nm, as F_1.

⑤ Incubate at 37°C for 10 min protected from light. Mix fully with fluorescence microplate for 5 s and measure the fluorescence intensity at the excitation wavelength of 328 nm and the emission wavelength of 393 nm, as F_2. (The standard curve is fitted to the standard well in 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:

Serum and plasma samples:

Definition: The amount of enzyme in 1 L serum or plasma per 1 min that produce 1 μmol chromogenic substance at 37 °C is defined as 1 unit.

$$\text{CatD activity (U/L)} = (\Delta F - b) \div a \times f \div T$$

Tissue and cell samples:

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

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

[Note]

ΔF: $\Delta F = F_2 - F_1$.

T: Reaction time, 10 min.

C_{pr}: Concentration of protein in sample, gprot/L

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse serum 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)	5.00	15.00	25.00
%CV	2.3	3.1	2.8

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.00	15.00	25.00
%CV	3.6	4.7	3.9

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 99.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc(U/L)	5.00	15.00	25.00
Observed Conc(U/L)	4.90	15.00	24.95
Recovery rate (%)	98.0	100.0	99.8

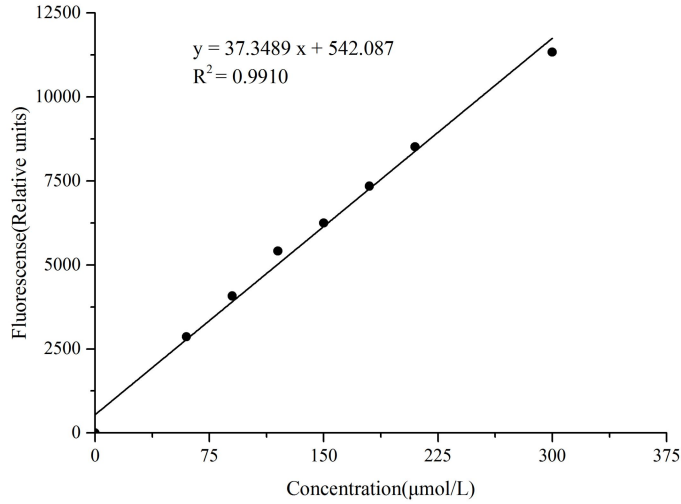
Sensitivity

The analytical sensitivity of the assay is 0.02 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	60	90	120	150	180	210	300
F_2 value	1919	4650	5722	7473	8141	9187	10407	13170
	1758	4751	6111	7037	8031	9178	10297	13171
Average F_2 value	1838	4700	5917	7255	8086	9183	10352	13171
Absoluted F_2 value	0	2862	4078	5416	6247	7344	8514	11333



Appendix II Example Analysis

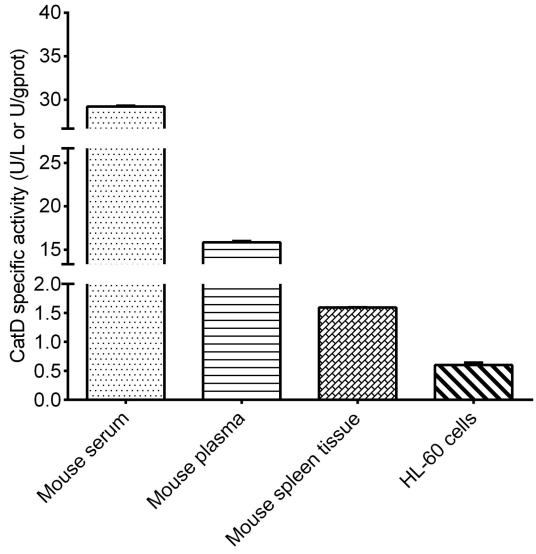
Example analysis:

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

Standard curve: y = 37.3489 x + 542.087, the average F_1 value of the sample well is 2372, the average F_2 value of the sample well is 9928, $\Delta F = F_2 - F_1 = 9928 - 2372 = 7556$, the concentration of protein is 11.78 gprot/L, and the calculation result is:

$$\text{CatD activity (U/gprot)} = (7556 - 542.087) \div 37.3489 \div 11.78 \div 10 = 1.59 \text{ U/gprot}$$

Detect mouse serum, mouse plasma, 10% mouse spleen tissue homogenate (the concentration of protein is 11.78 gprot/L) and 1×10^6 HL-60 cells (the concentration of protein is 2.18 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.