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

Catalog No：E-BC-K886-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader (730-770 nm)

Detection range：0.004-0.50 U/L

Elabscience® Protease Activity Colorimetric Assay Kit

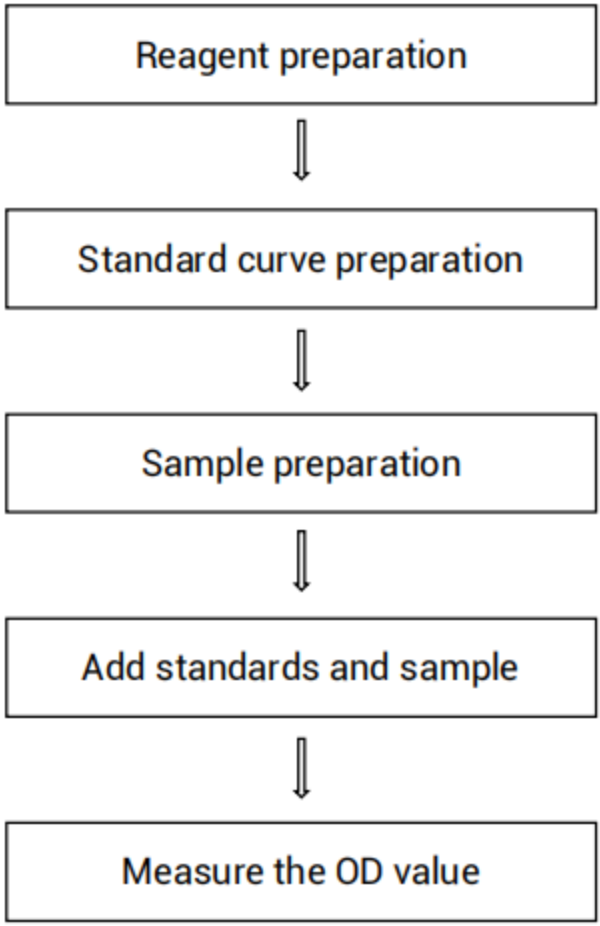
This manual must be read attentively and completely before using this product. If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can be used to detect the protease activity in animal and plant tissue samples.

Detection principle

Protease is a class of enzymes capable of catalyzing the hydrolysis of protein peptide bonds and belongs to the hydrolase family. They break down the substrate into smaller peptides or free amino acids by breaking the amide bonds (-CO-NH-) in proteins or peptides. Proteases are widely present in living organisms and are involved in various physiological processes, such as digestion, cell signal transduction, immune response and apoptosis.

The detection principle of this kit is: Protease hydrolyzes substrates to generate products. The activity of protease in the sample can be calculated by measuring the absorbance change of the product per unit time at a wavelength of 750 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	55 mL × 2 vials	2-8°C, 12 months
Reagent 2	Stop Solution	20 mL × 1 vial	2-8°C, 12 months
Reagent 3	Substrate	Powder × 2 vials	2-8°C, 12 months
Reagent 4	Substrate Solvent	1.6 mL × 2 vials	2-8°C, 12 months
Reagent 5	Chromogenic Agent A	24 mL × 1 vial	2-8°C, 12 months
Reagent 6	Chromogenic Agent B	1.2 mL × 2 vials	2-8°C, 12 months shading light
Reagent 7	50 mmol/L Standard Solution	1.6 mL × 1 vial	2-8°C, 12 months shading light

	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:

Microplate reader (730-770 nm, optimum wavelength: 750 nm), Incubator

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of substrate working solution:
Dissolve one vial of substrate with 1.2 mL of substrate solvent, mix well to dissolve. Then add 4.8 mL of extraction solution, mix fully. Store at 2-8°C for 7 days protected from light.

③ The preparation of chromogenic working solution:
Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 60 μL of chromogenic working solution (mix well 20 μL of chromogenic agent B and 40 μL of double distilled water). Keep it on ice during use protected from light and used up within 8 h.

④ The preparation of 2 mmol/L standard solution:

Before testing, please prepare sufficient 2 mmol/L standard solution. For example, prepare 1000 μL of 2 mmol/L standard solution (mix well 40 μL of 50 mmol/L standard solution and 960 μL of double distilled water). Store at 2-8°C for 7 days protected from light.

⑤ The preparation of standard curve：

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

Dilute 2 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.4, 0.8, 1, 1.2, 1.4, 1.6, 2 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.4**	**0.8**	**1**	**1.2**	**1.4**	**1.6**	**2**
2 mmol/L Standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Tissue samples:

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 in the same day.

④ Meanwhile, determine the protein concentration of supernatant (animal tissue:E-BC-K318-M; plant tissue:E-BC-K168-M).

② Dilution of sample

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

Sample type	Dilution factor
10% Mouse gastric tissue homogenate	1
10% Rat gastric tissue homogenate	1
10% Mouse intestinal tissue homogenate	1
10% Papaya flesh tissue homogenate	1
10% Pineapple flesh tissue homogenate	1
10% Kiwi fruit flesh tissue homogenate	1

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

Operating steps

Enzymatic reaction

① Sample tube: Add 50 μL of sample to the 2 mL EP tubes.
Control tube: Add 50 μL of sample to the 2 mL EP tubes.

② Add 100 μL of substrate working solution to sample tubes. Add 100 μL of extraction solution to control tubes.

③ Mix fully and incubate at 37°C for 10 min protected from light.

④ Add 150 μL of stop solution to each tube, mix fully. Centrifuge at 12000×g for 15 min at 4°C to remove insoluble material. Collect supernatant for detection.

Chromogenic reaction

① Standard well: Add 20 μL of standard solution with different concentrations into the corresponding wells.
Sample well: Add 20 μL of sample supernatant which was collected in sample tube after the reaction of the enzymatic reaction step into the corresponding wells.
Control well: Add 20 μL of sample supernatant which was collected in control tube after the reaction of the enzymatic reaction step into the corresponding wells.

② Add 200 μL of chromogenic agent A to each well.

③ Add 50 μL of chromogenic working solution to each well.

④ Mix fully and incubate at 37°C for 10 min protected from light. Measure the OD value of each well at 750 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD 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:

Tissue samples:

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

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

[Note]

ΔA: $\Delta A = OD_{sample} - OD_{control}$.

T: Reaction time, 10 min.

f: Dilution factor of sample before test.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse gastric tissue samples 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)	0.68	0.79	0.85
%CV	4.4	1.3	2.5

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.22	0.37	0.43
%CV	3.7	7.6	7.9

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 99.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.68	0.79	0.85
Observed Conc. (U/L)	0.7273	0.7900	0.7825
Recovery rate (%)	107	100	92

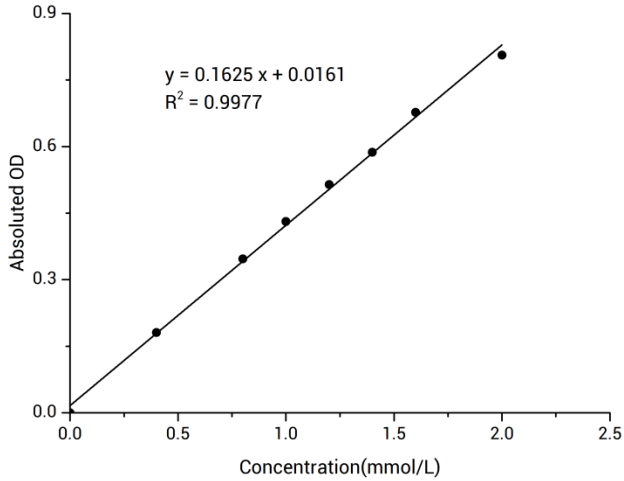
Sensitivity

The analytical sensitivity of the assay is 0.004 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 OD 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 (mmol/L)	0	0.4	0.8	1	1.2	1.4	1.6	2
OD value	0.050	0.229	0.395	0.483	0.563	0.641	0.729	0.859
	0.050	0.233	0.398	0.478	0.564	0.632	0.725	0.852
Average OD	0.050	0.231	0.397	0.481	0.564	0.637	0.727	0.856
Absoluted OD	0	0.181	0.347	0.431	0.514	0.587	0.677	0.806



Appendix Π Example Analysis

Example analysis：

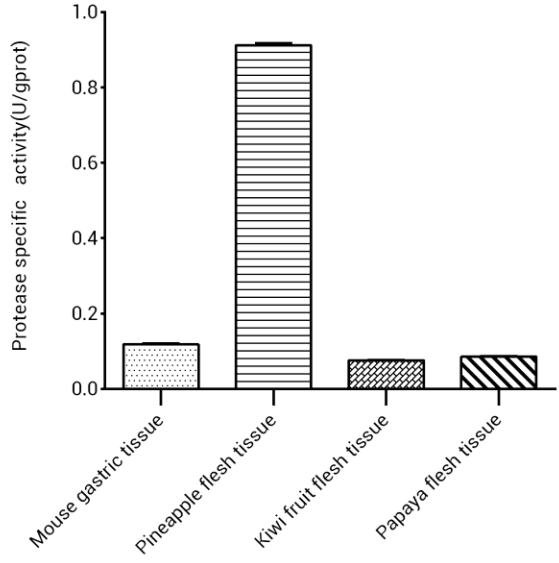
Take 50 μL of 10% pineapple flesh tissue homogenate and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.1625 x + 0.0161. The average OD value of sample well is 0.662, the average OD value of control well is 0.082, the concentration of protein is 0.38 gprot/L, and the calculation result is:

protease activity (U/gprot) = (0.662 – 0.082 - 0.0161) ÷ 0.1625 ÷ 0.38 ÷ 10

= 0.91 U/gprot

Detect 10% mouse gastric tissue homogenate (the concentration of protein is 2.81 gprot/L), 10% pineapple flesh tissue homogenate (the concentration of protein is 0.38 gprot/L), 10% kiwi fruit flesh tissue homogenate (the concentration of protein is 1.05 gprot/L) and 10% papaya flesh tissue homogenate (the concentration of protein is 0.40 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.