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

Catalog No：E-BC-K845-M

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

Measuring instrument：Microplate reader (400-410 nm)

Detection range：0.028-1.01 U/L

Elabscience® Chymotrypsin 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.

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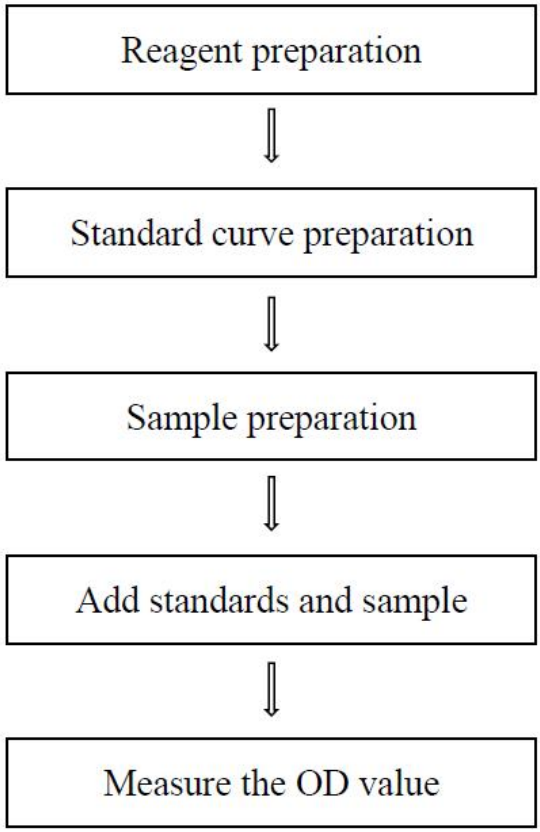
Assay summary



Intended use

This kit can measure chymotrypsin activity in tissue samples.

Detection principle

Chymotrypsin is a serine trypsin secreted by the pancreas, which is mainly used for the decomposition of proteins during digestion. It specifically hydrolyzes peptide bonds of aromatic amino acids (such as phenylalanine, tryptophan, and tyrosine) in proteins to generate smaller peptides or amino acids for further absorption by the body. In addition, chymotrypsin is widely used in protein analysis and research in the laboratory, especially for protein sequencing and structural analysis.

The detection principle of this kit is: The substrate was converted to a colored substance after chymotrypsin catalysis, and the OD value was detected at a wavelength of 405 nm, and the enzyme activity was calculated from the OD value of the standard.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	50 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Substrate	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	5 mmol/L Standard Solution	1.5 mL × 1 vial	1.5 mL × 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	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:

Microplate reader (400-410 nm, optimum wavelength: 405 nm), Incubator(37℃)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of working solution:

Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 200 μL of working solution (mix well 8 μL of substrate and 192 μL of buffer solution). Keep it on ice during use protected from light and used up within same day.

③ The preparation of 1 mmol/L standard solution:

Before testing, please prepare sufficient 1 mmol/L standard solution. For example, prepare 1000 μL of 1 mmol/L standard solution (mix well 200 μL of 5 mmol/L standard solution and 800 μL of buffer solution). The 1 mmol/L standard solution should be prepared on spot and store protected from light.

④ The preparation of standard curve:

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

Dilute 1 mmol/L standard solution with buffer solution diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8, 1 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.7**	**0.8**	**1**
1 mmol/L standard (μL)	0	40	60	80	120	140	160	200

Buffer solution (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation

Tissue sample:

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

② Wash tissue in normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

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

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
10% Mouse small intestine tissue homogenate	40-50
10% Mouse pancreas tissue homogenate	5-10
10% Mouse liver tissue homogenate	5-10

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 Standard well: Add 20 μL of standard solution with different concentrations to the corresponding wells.
Sample well: Add 20 μL of samples to sample wells.

② Add 180 μL of working solution to each well.

③ Mix fully with microplate reader for 5 s and measure the OD value of each well at 405 nm, as A_1. Incubate at 37°C for 30 min and measure the OD value of each well at 405 nm, as A_2. The standard curve is fitted to the standard well in A_2 value.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean A_2 value of the blank (Standard #①) from all standard readings. This is the absoluted A_2 value.

3. Plot the standard curve by using absoluted A_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:

Tissue sample:

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

$$\text{chymotrypsin activity (U/gprot)} = (\Delta A_{405} - b) \div a \div T \times f \div C_{pr} \times 1000$$

[Note]

ΔA_{405}: $\Delta A_{405} = A_2 - A_1$.

T: Reaction time, 30 min.

f: Dilution factor of sample before test.

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

1000: 1 mmol/L = 1000 μmol/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver 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)	0.20	0.40	0.80
%CV	1.8	2.2	4.0

Inter-assay Precision

Three mouse liver 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)	0.20	0.40	0.80
%CV	1.2	6.1	9.7

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%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.20	0.40	0.80
Observed Conc. (U/L)	0.19	0.40	0.82
Recovery rate (%)	95.0	99.0	102.0

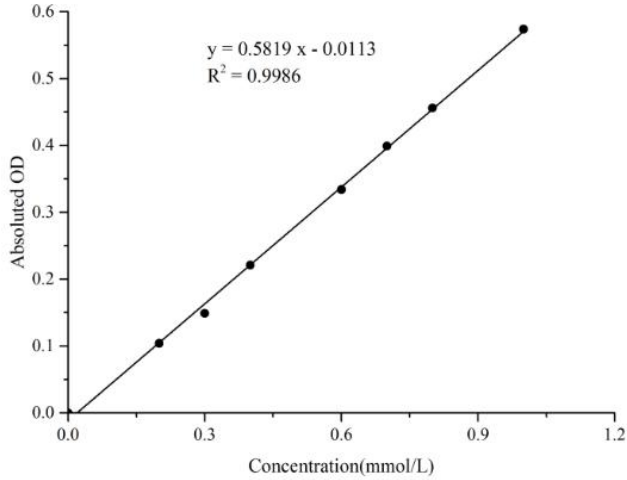
Sensitivity

The analytical sensitivity of the assay is 0.028 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**	**0.2**	**0.3**	**0.4**	**0.6**	**0.7**	**0.8**	**1**
A_2 value	0.077	0.182	0.226	0.302	0.415	0.475	0.536	0.639
	0.078	0.182	0.227	0.299	0.412	0.477	0.534	0.652
Average A_2 value	0.078	0.182	0.227	0.300	0.414	0.476	0.535	0.646
Absoluted A_2 value	0	0.104	0.149	0.221	0.334	0.399	0.456	0.574



Appendix II Example Analysis

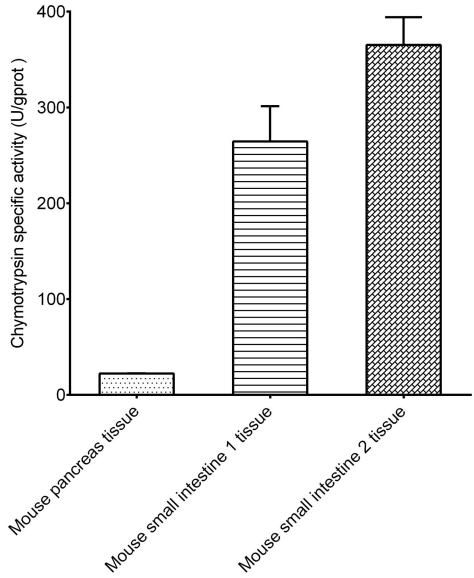
Example analysis:

Take 20 μL of 10% mouse small intestine tissue homogenate which dilute for 50 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y =0.5819 x – 0.0113, the A_1 of the sample well is 0.107, the A_2 of the sample well is 0.521, ΔA_{405} = 0.521 - 0.107 = 0.414, the concentration of protein is 4.97 gprot/L, and the calculation result is:

$$\text{chymotrypsin activity (U/gprot)} = (0.414 + 0.0113) \div 0.5819 \div 30 \times 50 \div 4.97 \times 1000$$
$$= 245.1 \text{ U /gprot}$$

Detect 10% mouse pancreas tissue homogenate (the concentration of protein is 13.18 gprot/L, dilute for 5 times), 10% mouse small intestine tissue 1 homogenate (the concentration of protein is 4.97 gprot/L, dilute for 50 times) and 10% mouse small intestine tissue 2 homogenate (the concentration of protein is 7.27 gprot/L, dilute for 50 times) 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.