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

Catalog No：E-BC-K843-M

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

Measuring instrument：Microplate reader（400-410 nm）

Detection range：1.86-100.65 U/L

Elabscience®Trypsin Activity 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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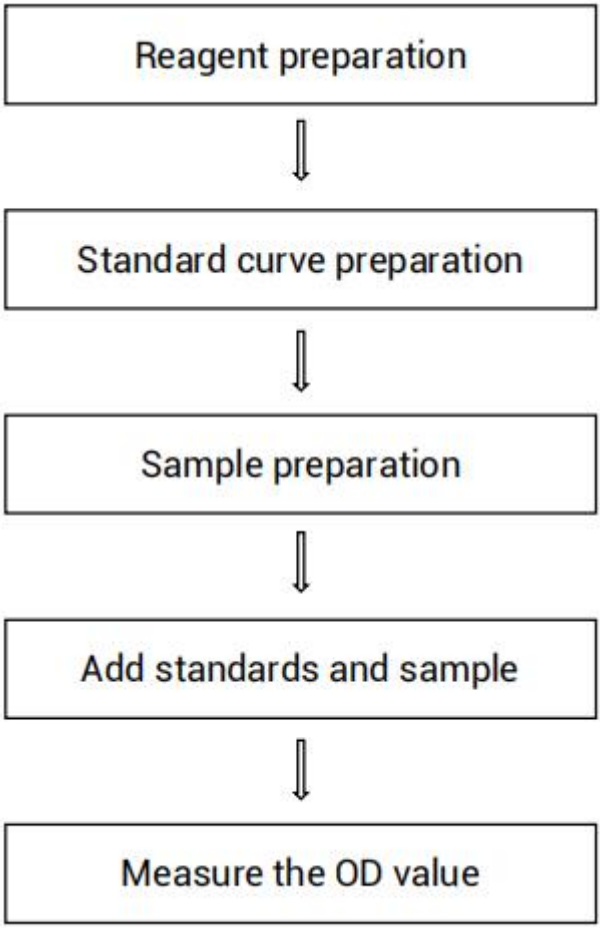
Assay summary



Intended use

This kit can be used to measure Trypsin activity in animal tissue samples.

Detection principle

Trypsin catalyzes the substrate to form p-nitroaniline, which has a certain light absorption value at 400-410 nm wavelength. Since the absorbance of p-NA is proportional to the content, the activity of trypsin can be calculated by measuring the p-NA produced per unit time.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	50 mL × 2 vials	-20℃, 12 months
Reagent 2	Substrate	Power × 1 vial	Power × 2 vials	-20℃, 12 months, shading light
Reagent 3	Standard	Power × 1 vial	Power × 2 vials	-20℃, 12 months, shading light
Reagent 4	Diluent	2 mL × 1 vial	4 mL × 1 vial	-20℃, 12 months
	Microplate	48 wells	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 (400-410 nm, optimum wavelength: 405 nm), Incubator

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate working solution：
Dissolve one vial of substrate with 0.5 mL of diluent, mix well to dissolve. The prepared solution should be used up within 4 hours.

③ Preparation of reaction working solution：
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 250 μL of reaction working solution (mix well 240 μL of buffer solution and 10 μL of substrate working solution). The reaction working solution should be prepared on spot.

④ Preparation of standard working solution：
Dissolve one vial of standard with 1 mL of diluent, mix well to dissolve. Store at -20°C for 7 days protected from light.

⑤ Preparation of 1 mmol/L standard solution：
Before testing, please prepare sufficient 1 mmol/L standard solution according to the test wells. For example, prepare 1000 μL of 1 mmol/L standard solution (mix well 50 μL of standard working solution and 950 μL of buffer solution). The 1 mmol/L standard solution should be prepared on spot and should be used up within 4 hours.

⑥ 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 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**
10 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 cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL buffer solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes 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	2-5
10% Mouse large intestine tissue homogenate	2-5
10% Rat intestine tissue homogenate	2-5
10% Rat chyme tissue homogenate	2-5

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

Operating steps

① Standard well: Add 10 μL of standards with different concentrations to the corresponding wells.

Sample well: Add 10 μL of sample to the corresponding wells.

② Add 160 μL of reaction working solution to standard well and sample well.

③ Mix fully for 5 s with microplate reader. Measure the OD value of sample well at 405 nm, recorded as A_1.

④ Incubate at 37℃ for 10 min, measure the OD value of each well at 405 nm, recorded as A_2.

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 sample:

Definition: The amount of trypsin in 1 g tissue protein per minute that catalyze the substrate to produce 1 μmol p-NA at 37℃ is defined as 1 unit

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

[Note]

ΔA_{405}: Absolute OD ($A_2 - A_1$).

T: The time of reaction, 10 min.

f: Dilution factor of sample before test.

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

1000: 1 mmol/L=1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat intestine 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)	5.60	26.80	76.40
%CV	5.1	3.9	4.5

Inter-assay Precision

Three rat intestine 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)	5.60	26.80	76.40
%CV	11.2	7.2	9.8

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 106%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.24	0.5	0.73
Observed Conc. (mmol/L)	0.3	0.5	0.8
Recovery rate (%)	113	100	105

Sensitivity

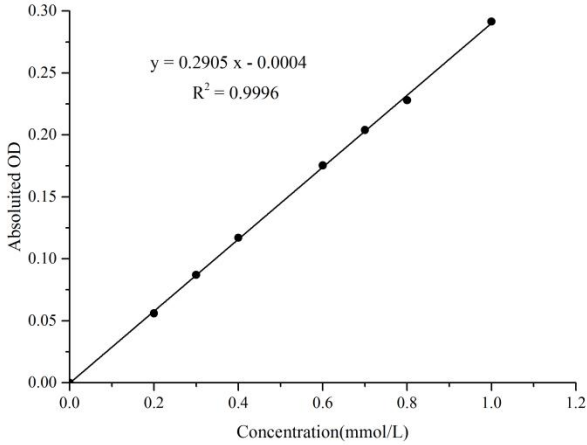
The analytical sensitivity of the assay is 1.86 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding

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**	**1.0**
Average OD	0.367	0.423	0.454	0.484	0.543	0.571	0.595	0.659
Absoluted OD	0	0.056	0.087	0.117	0.176	0.204	0.228	0.292



Appendix Π Example Analysis

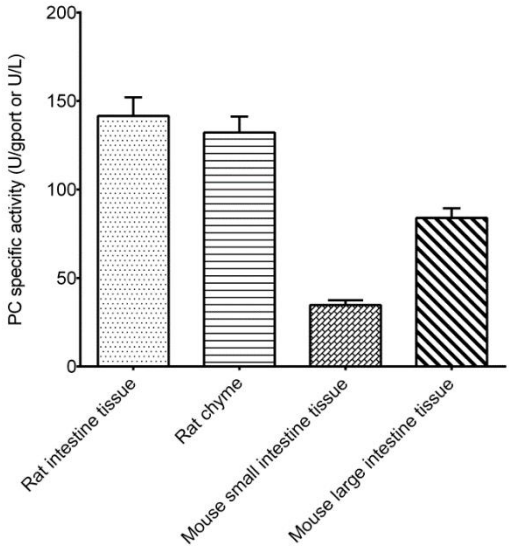
Example analysis：

For 10% mouse small intestine tissue homogenate, dilute for 2.5 times, take 10 μL and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.2905x - 0.0004, the A_1 of sample is 0.555, the A_2 of sample is 0.606, $\Delta A_{405} = A_2 - A_1 = 0.606 - 0.555 = 0.051$, the concentration of protein in sample is 1.32 gprot/L, and the calculation result is:

$$\text{Trypsin activity (U/gprot)} = (0.051 + 0.0004) \div 0.2905 \div 10 \times 2.5 \div 1.324 \times 1000 = 33.42 \text{ U/gprot}$$

Detect 10% mouse small intestine tissue homogenate (the concentration of protein is 1.32 gprot/L), 10% mouse large intestine tissue homogenate (the concentration of protein is 1.22 gprot/L), 10% rat intestine tissue homogenate (the concentration of protein is 0.98 gprot/L) and 10% rat chyme tissue homogenate (the concentration of protein is 1.76 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.