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

Catalog No：E-BC-K844-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（650-670 nm）

Detection range：0.63-94.98 U/mL

Elabscience®Pepsin 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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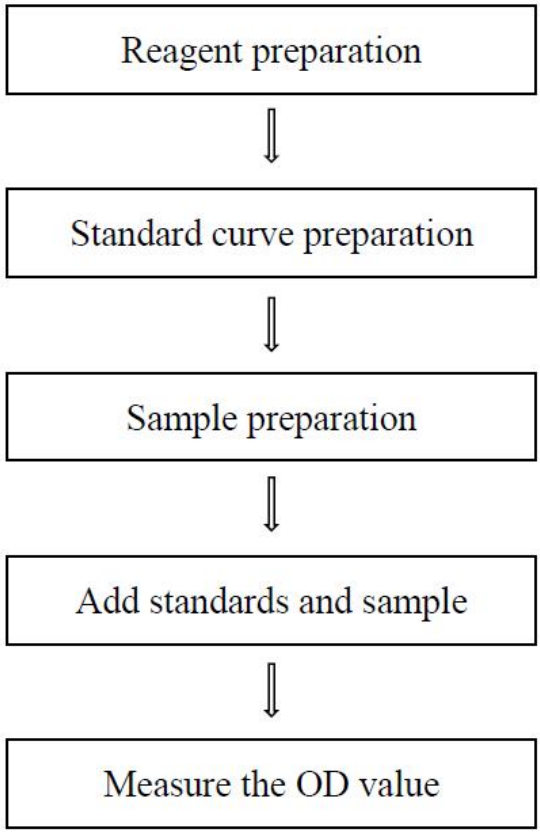
Assay summary



Intended use

This kit can measure pepsin activity in gastric, intestinal tissue samples.

Detection principle

Pepsin can catalyze substrate hydrolysis, and the hydrolysate reacts with the chromogenic agent under alkaline conditions to form a blue substance. The blue substance has a maximum absorption peak at 660 nm, and the color depth is proportional to the activity of pepsin. The enzyme activity can be calculated by OD value.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	55 mL × 2 vials	-20℃, 12 months
Reagent 2	Precipitating Agent	50 mL × 1 vial	-20℃, 12 months
Reagent 3	Chromogenic Agent A	20 mL × 1 vial	-20℃, 12 months
Reagent 4	Chromogenic Agent B	10 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	Substrate	Power×2 vials	-20℃, 12 months, shading light
Reagent 6	Substrate Solvent	12 mL × 1 vial	-20℃, 12 months
Reagent 7	1 mg/mL Standard Solution	1.5 mL × 1 vial	-20℃, 12 months, shading light
	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:

Microplate reader (650-670 nm, optimum wavelength: 660 nm)，Incubator

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate working solution：

Dissolve one vial of substrate with 5 mL of substrate solvent. Dissolve completely in 75°C water bath for 30 min. Store at 2-8°C for 2 days.

③ Preparation of 60 μg/mL standard solution：

Before testing, please prepare sufficient 60 μg/mL standard solution according to the test wells. For example, prepare 1000 μL of 60 μg/mL standard solution (mix well 940 μL of buffer solution and 60 μL of 1 mg/mL standard solution). The prepared solution should be prepared on spot.

④ Preparation of measuring working solution：

Before testing, please prepare sufficient measuring working solution according to the test wells. For example, prepare 320 μL of measuring working solution (mix well 195 μL of buffer solution and 125 μL of substrate working solution). The prepared solution should be prepared on spot.

⑤ The preparation of standard curve：

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

Dilute 60 μg/mL standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 12, 18, 24, 36, 42, 48, 60 μg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μg/mL)	**0**	**12**	**18**	**24**	**36**	**42**	**48**	**60**
60 μg/mL 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 30 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 30 mg tissue in 120 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
20% Rat gastric tissue homogenate	1
20% Mouse gastric tissue homogenate	1
20% Rat intestinal tissue homogenate	1
20% Mouse intestinal tissue homogenate	1

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

Operating steps

Enzymatic reaction

① Control tube: Take 40 μL of sample to the 2 mL EP tube.
Sample tube: Take 40 μL of sample to the 2 mL EP tube.

② Add 400 μL of precipitating agent to control tube.

③ Add 200 μL of measuring working solution to control tube and sample tube. Mix fully and incubate at 37℃ for 10 min

④ Add 400 μL of precipitating agent to sample tube.

⑤ Mix fully and incubate at 37℃ for 10 min, then centrifuge at 3500 r/min for 10 min and take the supernatant for detection.

Chromogenic reaction

① Standard well: Take 60 μL of standards with different concentrations to the corresponding wells.
Control well: Take 60 μL of supernatant of control tube to the corresponding wells.
Sample well: Take 60 μL the supernatant of sample tube to the corresponding wells.

② Add 150 μL of chromogenic agent A to each well.

③ Add 60 μL of chromogenic agent B to each well

④ Mix fully with microplate reader and incubate at 37℃ for 10 min. Measure the OD value of each well at 660 nm with microplate reader

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 pepsin in 1 mg tissue protein per 1 min that catalyze of substrate to produce 1 μg product at 37°C is defined as 1 unit.

$$\text{Pepsin activity (U/mgprot)} = (\Delta A_{660} - b) \div a \div T \times (V_1 \div V_2) \div C_{pr} \times f$$

[Note]

ΔA_{660}: $(OD_{Sample} - OD_{Control})$.

T: The time of enzymatic reaction, 10 min.

V_1: The volume of enzymatic reaction, 0.64 mL.

V_2: The volume of sample in enzymatic reaction, 0.04 mL.

C_{pr}: The concentration of protein in sample, mgprot/mL.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat 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/mL)	3.80	46.80	73.50
%CV	2.2	1.6	1.3

Inter-assay Precision

Three rat 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/mL)	3.80	46.80	73.50
%CV	4.8	5.6	5.2

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 95%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μg/mL)	15	32	45.5
Observed Conc. (μg/mL)	14.7	29.8	42.8
Recovery rate (%)	98	93	94

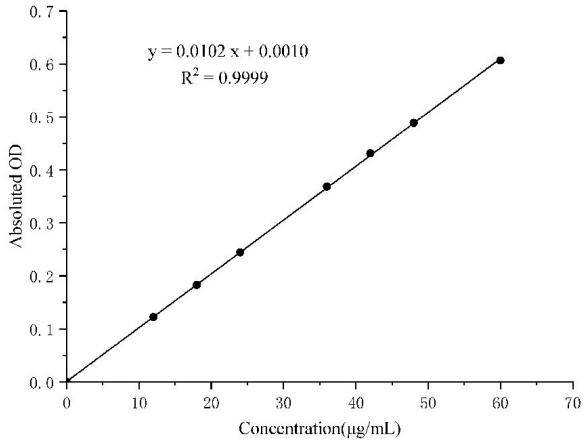
Sensitivity

The analytical sensitivity of the assay is 0.63 U/mL. 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 (μg/mL)	0	12	18	24	36	42	48	60
OD Value	0.041	0.163	0.222	0.285	0.411	0.476	0.530	0.647
	0.041	0.164	0.226	0.286	0.408	0.469	0.529	0.648
Average OD	0.041	0.164	0.224	0.286	0.410	0.473	0.530	0.648
Absoluted OD	0	0.123	0.183	0.245	0.369	0.432	0.489	0.607



Appendix II Example Analysis

Example analysis：

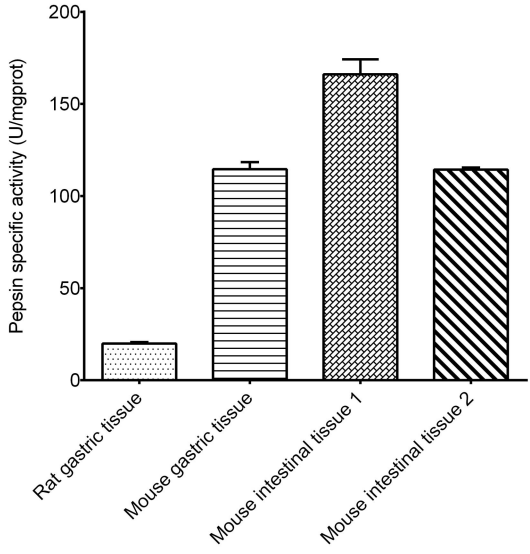
For 20% rat gastric tissue homogenate, take 40 μL of sample and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0102 x + 0.001, the average OD value of the control is 0.379, the average OD value of the sample is 0.414, the concentration of protein in sample is 0.268 mgprot/mL, and the calculation result is:

Pepsin activity (U/mgprot) = (0.414 – 0.379 – 0.001) ÷ 0.0102 ÷ 10 × 1 × (0.64 ÷ 0.04) ÷ 0.268

= 19.9 U/mgprot

Detect 20% rat gastric tissue homogenate (the concentration of protein is 0.268 mgprot/mL), 20% mouse gastric tissue homogenate (the concentration of protein is 0.251 mgprot/mL), 20% mouse intestinal tissue 1 homogenate (the concentration of protein is 0.216 mgprot/mL), 20% mouse intestinal tissue 2 homogenate (the concentration of protein is 0.281 mgprot/mL) 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.