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

Catalog No：E-BC-K899-M

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

Measuring instrument：Microplate reader(370-390 nm)

Detection range：0.54-28.57 U/L

Elabscience® Neutrophil Elastase (NE) 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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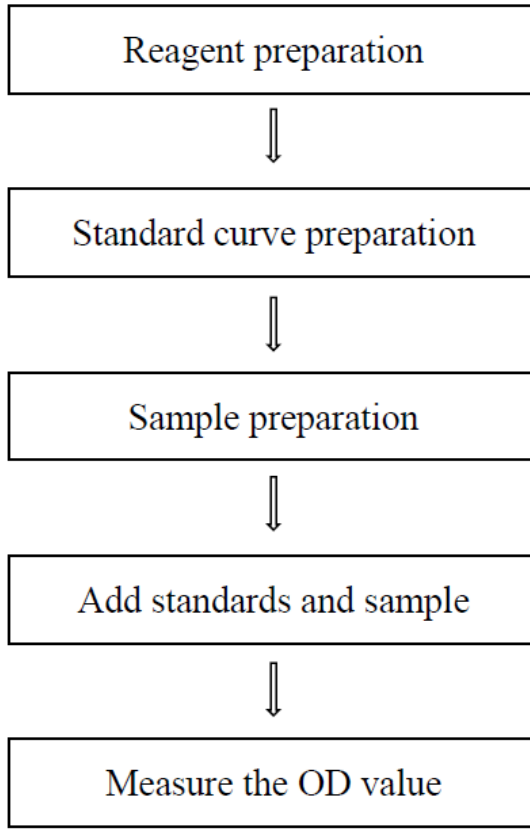
Assay summary



Intended use

This kit can be used to measure neutrophil elastase (NE) activity in animal tissue sample.

Detection principle

Neutrophil Elastase (NE) is one of the main serine proteases secreted by neutrophils. It plays an important role in the physiological and pathological processes of the body, on the one hand NE participate in the formation of the body's defense system and assist in the elimination of pathogenic microorganisms. On the other hand, the excessive activation of NE, in the case of imbalance with endogenous inhibitors, is directly involved in tissue damage, and is closely related to the occurrence of a variety of inflammation-related diseases, such as atherosclerosis, chronic obstructive pulmonary disease, pulmonary cystic fibrosis and other diseases, which can be used as a prevention and treatment target for these diseases.

The detection principle of this kit is as follows: NE hydrolyzates the substrate to generate chromogenicity substances, which have maximum absorption at the wavelength of 380 nm, and NE activity in the sample is calculated by measuring the OD value at 380 nm and the standard curve.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	40 mL ×1 vial	40 mL ×2 vials	-20℃, 12 months
Reagent 2	Buffer Solution	10 mL ×1 vial	20 mL ×1 vial	-20℃, 12 months, shading light
Reagent 3	Substrate	0.42 mL×1 vial	0.84 mL×1 vial	-20℃, 12 months, shading light
Reagent 4	Reducing Reagent	0.55 mL×1 vial	1.1 mL×1 vial	-20℃, 12 months, shading light
Reagent 5	Stop Solution	1.1 mL×1 vial	2.2 mL×1 vial	-20℃, 12 months, shading light
Reagent 6	10 mmol/L Standard Solution	0.1 mL×1 vial	0.1 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 (370-390 nm, optimum wavelength: 380 nm), Incubator (37℃)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use. Centrifuged the substrate, reducing reagent and 10 mmol/L standard solution to the bottom of the tube before use.

② The preparation of substrate working solution:

For each well, prepare 150 μL of substrate working solution (mix well 7.5 μL of substrate and 142.5 μL of buffer solution). The substrate working solution should be prepared on spot. The prepared solution should be used up within 4 h.

③ The 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 100 μL of 10 mmol/L standard solution and 900 μL of buffer solution). Store at 2-8 ℃ for a month.

④ The preparation of standard curve：

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

Dilute 1 mmol/L standard with buffer solution to a serial concentration, the recommended dilution gradient is 0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 1.0 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**	**0.5**	**0.6**	**0.7**	**1.0**
1 mmol/L standard (μL)	0	40	60	80	100	120	140	200
Buffer solution (μL)	200	160	140	120	100	80	70	0

Sample preparation

① Sample preparation

Tissue sample:

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

② Homogenize 20 mg tissue in 180 μL extracting solution with a dounce homogenizer at 4℃.

③ Centrifuge at 12000 × g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ 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 Pancreas tissue homogenate	5-15
10% Mouse Small intestine tissue homogenate	1
10% Mouse Stomach tissue homogenate	1

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

The key points of the assay

Extracting solution, buffer solution and stop solution are easy to produce foam during the suction and beating process, so it is recommended to be careful when using the pipetting gun.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into the corresponding wells.

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

② Add 10 μL of reducing reagent into each well.

③ Add 150 μL of substrate working solution into each well.

④ Mix fully with microplate reader for 5 s. Measure the OD value of each well at 380 nm with microplate reader, as A_1.

⑤ Incubate at 37 ℃ for 35 min protected from light.

⑥ Add 20 μL of stop solution into each well. Mix fully with microplate reader for 5 s. Measure the OD value of each well at 380 nm with microplate reader, 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 enzyme in 1 g tissue protein that catalyze the substrate to 1 μmol glycerol 3-phosphate in 1 min at 37℃ is defined as 1 unit.

$$\underset{\text{(U/gprot)}}{\text{NE activity}} = (\Delta A - b) \div a \div C_{pr} \times f \div T \times 1000^{*}$$

[Note]

ΔA: $\Delta A = A_2 - A_1$.

f: Dilution factor of sample before test.

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

1000*: 1 mmol/L=1000 μmol/L.

T: Reaction time, 35 min.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse pancreas 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）	5.00	10.00	20.00
%CV	4.1	5.0	4.5

Inter-assay Precision

Three mouse pancreas 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)	5.00	10.00	20.00
%CV	6.3	7.5	6.9

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%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	5.00	10.00	20.00
Observed Conc. (U/L)	4.90	10.00	19.70
recovery rate(%)	98	100	99

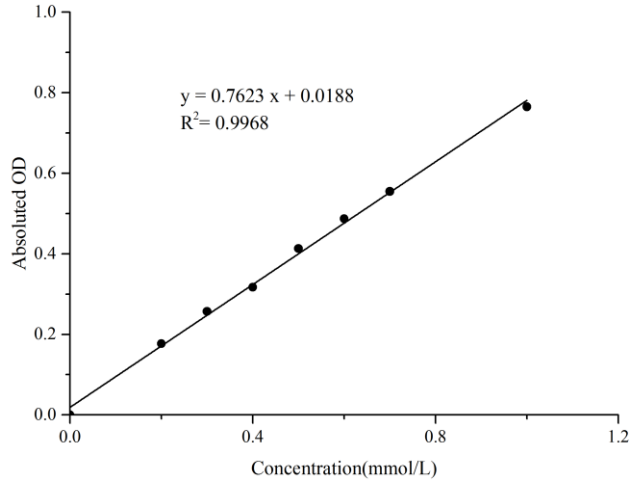
Sensitivity

The analytical sensitivity of the assay is 0.54 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**	**0.5**	**0.6**	**0.7**	**1.0**
A_2	0.270	0.450	0.543	0.607	0.683	0.726	0.820	1.054
	0.270	0.444	0.511	0.568	0.683	0.789	0.831	1.016
Average A_2	0.271	0.447	0.527	0. 588	0.683	0.758	0.826	1.035
OD	0	0.177	0.257	0.317	0.413	0.487	0.555	0.765



Appendix Π Example Analysis

Example analysis:

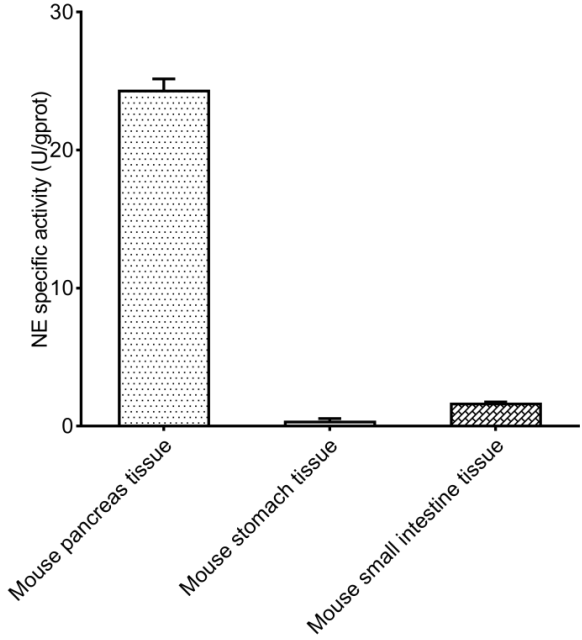
Take 20 μL of 10% mouse pancreas tissue homogenate (dilute for 5 times) and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.7623 x + 0.0188, the A_1 value of the sample well is 0.383, the A_2 value of the sample well is 1.120, $\Delta A = A_2 - A_1 = 1.120 - 0.383 = 0.737$, the concentration of protein is 5.70 gprot/L, and the calculation result is:

$$\text{NE activity (U/gprot)} = (0.737 - 0.0188) \div 0.7623 \times 5 \div 35 \div 5.7 \times 1000$$

$$= 23.61 \text{ U/gprot}$$

Detect mouse serum, 10% mouse pancreas tissue homogenate (the concentration of protein is 5.7 gprot/L), 10% mouse stomach tissue homogenate (the concentration of protein is 4.12 gprot/L) and 10% mouse small intestine tissue homogenate (the concentration of protein is 7.43 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.