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

Catalog No：E-BC-K1224-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader (580-590 nm)

Detection range：5.63-55.4 U/L

Elabscience® Chitinase 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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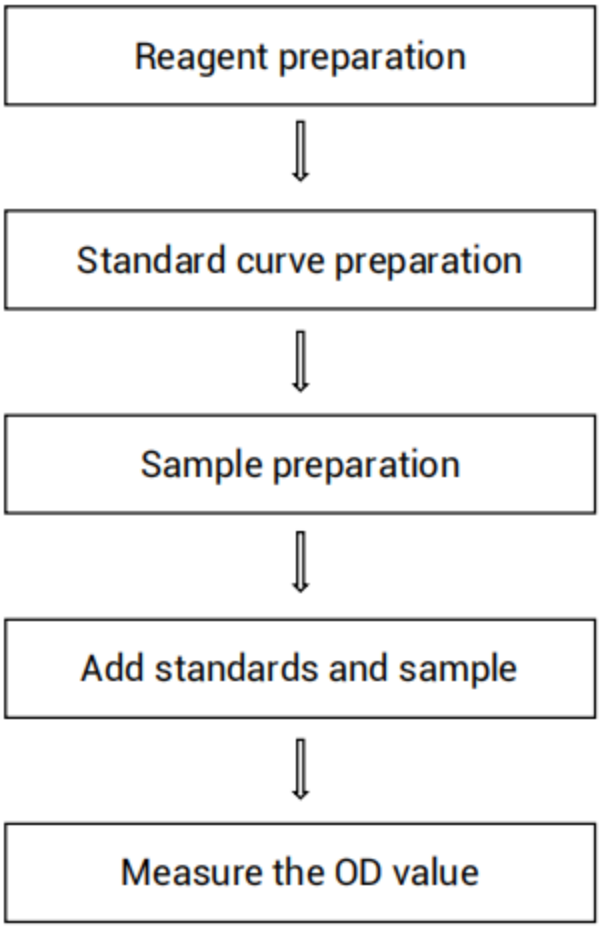
Assay summary



Intended use

This kit can be used to detect the chitinase activity in tissue and cell samples.

Detection principle

Chitinase is mainly present in various crustacean tissues, microorganisms and cells, and participates in a variety of physiological reaction processes. Chitinase can effectively degrade chitin, and thus plays a very important role in the material cycle of nature.

The detection principle of this kit: Chitinase can hydrolyze chitin. The hydrolysis product, which is heated with an alkali, can further react with the chromogenic reagent. The chromogenic substance generated has the maximum absorbance at a wavelength of 585 nm. The chitinase activity of the sample is calculated by measuring the absorbance.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	55 mL × 1 vial	-20°C, 12 months shading light
Reagent 2	Substrate	18 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Alkali Reagent	4 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Chromogenic Agent	40 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	Standard	Powder × 2 vials	-20°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 (580-590 nm, optimum wavelength: 585 nm), Incubator (37°C), Water bath

Reagent preparation

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

② The substrate is a suspension. If left standing for a long time, there will be sediment. It needs to be thoroughly mixed before use.

③ The preparation of 45 mmol/L standard solution:
Dissolve one vial of standard with 1 mL of buffer solution, mix well to dissolve. Store at -20°C for 7 days protected from light.

④ The preparation of 450 μmol/L standard solution:
Before testing, please prepare sufficient 450 μmol/L standard solution. For example, prepare 1000 μL of 450 μmol/L standard solution (mix well 10 μL of 45 mmol/L standard solution and 990 μL of buffer solution). Keep it on ice during use protected from light and used up within 8 h.

⑤ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 450 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 90, 135, 180, 270, 315, 360, 450 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**90**	**135**	**180**	**270**	**315**	**360**	**450**
450 μmol/L Standard (μL)	0	60	90	120	180	210	240	300
Buffer solution (μL)	300	240	210	180	120	90	60	0

Sample preparation

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 buffer 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.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell samples:

① Harvest the number of fungus needed for each assay (initial recommendation 1×10^6 cells).

② Homogenize 1×10^6 cells in 200 μL buffer 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.

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% Metapenaeus ensis shell tissue homogenate	2-5
1×10^6 HL-60 cells	1
1×10^6 A549 cells	1
1×10^6 K562 cells	1

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

The key points of the assay

When using the boiling water bath, holes need to be drilled in the EP tube cap to prevent it from popping off due to high temperature and affecting the measurement values.

Operating steps

Enzymatic reaction

① Sample tube: Add 80 μL of sample to the 2 mL EP tubes.
Control tube: Add 80 μL of sample to the 2 mL EP tubes.

② Add 160 μL of substrate to each tube.

③ Mix fully and incubate at 37°C for 60 min protected from light. Boiling water bath for 5 min, then cool to 25°C with running water. Centrifuge at 10000×g for 5 min to remove insoluble material. Collect supernatant for detection.

Chromogenic reaction

① Standard tube: Add 100 μL of standard solution with different concentrations into the corresponding tubes.
Sample tube: Add 100 μL of sample supernatant which was collected in sample tube after the reaction of the enzyme reaction step into the corresponding tubes.
Control tube: Add 100 μL of sample supernatant which was collected in control tube after the reaction of the enzyme reaction step into the corresponding tubes.

② Add 30 μL of alkali reagent to each tube.

③ Control tube: Mix fully and stand at 25°C for 5 min.
Standard tube/Sample tube: Mix well, boiling water bath for 5 min, then cool to 25°C with running water.

④ Add 350 μL of chromogenic agent to each tube.

⑤ Incubate at 37°C for 20 min protected from light. Collect 200 μL solution to corresponding wells. Measure the OD value of each well at 585 nm, recorded as A.

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 or cell samples:

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

$$\text{chitinase activity (U/gprot)} = (\Delta A_{585} - b) \div a \div T \times f \div C_{pr}$$

[Note]

ΔA_{585}: $\Delta A = A_{sample} - A_{control}$.

T: Reaction time, 60 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 1×10^6 HL-60 cells 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)	10.00	20.00	40.00
%CV	0.2	1.0	1.4

Inter-assay Precision

Three 1×10^6 HL-60 cells 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)	10.00	20.00	40.00
%CV	3.1	7.3	9.6

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)	10.00	20.00	40.00
Observed Conc. (U/L)	10.0	19.4	40.4
Recovery rate (%)	100	97	101

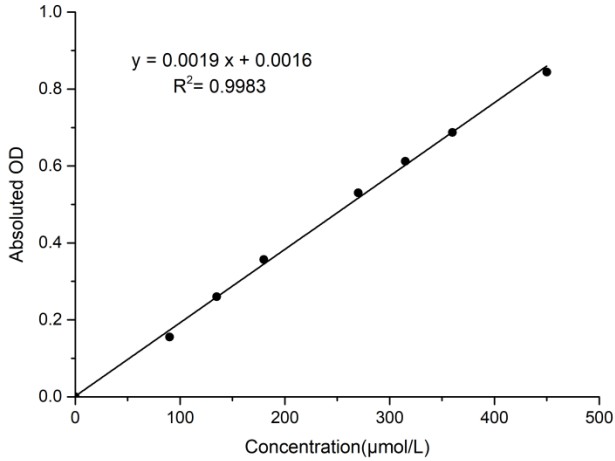
Sensitivity

The analytical sensitivity of the assay is 5.63 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 (μmol/L)	0	90	135	180	270	315	360	450
OD value	0.144	0.292	0.403	0.499	0.670	0.735	0.819	0.966
	0.144	0.306	0.404	0.501	0.678	0.776	0.843	1.009
Average OD	0.144	0.299	0.404	0.500	0.674	0.756	0.831	0.988
Absoluted OD	0.000	0.156	0.260	0.357	0.531	0.612	0.688	0.844



Appendix Π Example Analysis

Example analysis：

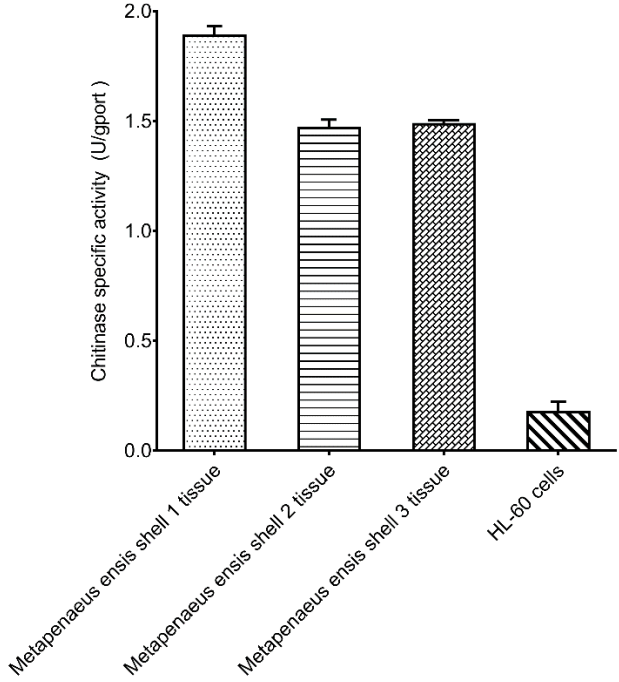
Take 80 μL of 10% metapenaeus ensis shell 1 tissue homogenate which dilute for 4 times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0019x + 0.0016. The OD value of sample well is 0.525, the OD value of control well is 0.164, $\Delta A_{585} = A_{sample} - A_{control}$ = 0.525 - 0.164 = 0.361, the concentration of protein is 6.76 gprot/L, and the calculation result is:

chitinase activity (U/gprot) = (0.361 – 0.0016) ÷ 0.0019 ÷ 60 × 4 ÷ 6.76

= 1.87 U/gprot

Detect 10% metapenaeus ensis shell 1 tissue homogenate (the concentration of protein is 6.76 gprot/L, dilute for 4 times), 10% metapenaeus ensis shell 2 tissue homogenate (the concentration of protein is 6.03 gprot/L, dilute for 4 times), 10% metapenaeus ensis shell 3 tissue homogenate (the concentration of protein is 6.61 gprot/L, dilute for 4 times) and 1×10^6 cells homogenate (the concentration of protein is 0.60 gprot/L), according to the protocol, the result is as follows：


Chitinase specific activity (U/gport)
2.0
1.5
1.0
0.5
0.0
Metapenaeus ensis shell 1 tissue
Metapenaeus ensis shell 2 tissue
Metapenaeus ensis shell 3 tissue
HL-60 cells

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.