

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

Catalog No: E-BC-K1405-M

Specification: 96T(39 samples)

Measuring instrument: Microplate reader(640-660 nm)

Detection range: 0.13-66.67 U/L

Elabscience® Urease 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

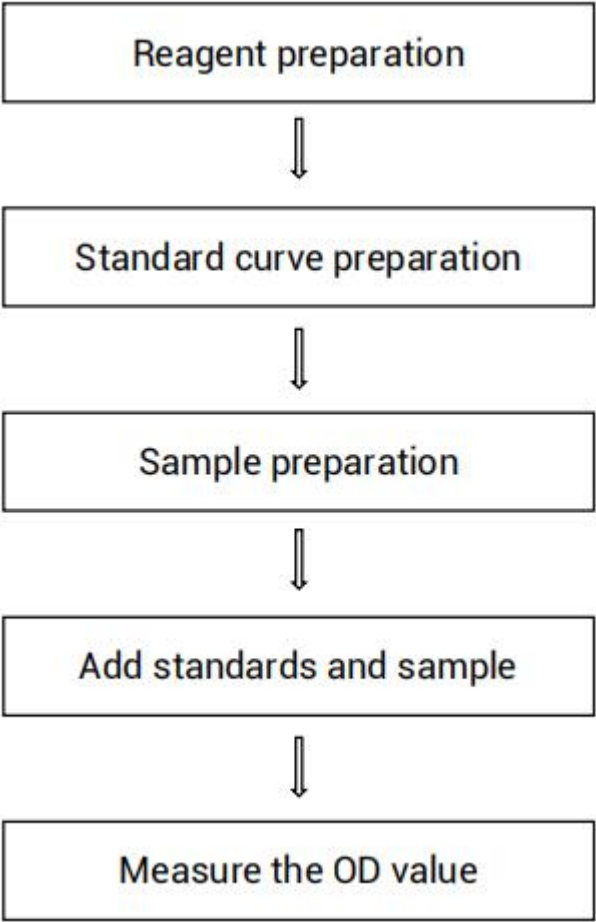
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary	3
Intended use	4
Detection principle	4
Kit components & storage	5
Instruments	5
Reagent preparation	6
Sample preparation	7
Operating steps	9
Calculation	10
Appendix I Performance Characteristics	12
Appendix II Example Analysis	14
Statement	16

Assay summary



Intended use

The kit is suitable for detecting the activity of urease in plant tissues and microorganism.

Detection principle

Urease, also known as urea amidohydrolase, is an oligomeric enzyme containing divalent nickel ions. It exhibits absolute specificity, specifically catalyzing the hydrolysis of urea to release ammonia and carbon dioxide, and plays an important role in the nitrogen cycle in nature. Urease is mainly extracted from the seeds of leguminous plants such as jack beans and soybeans, and microorganisms such as *Arthrobacter* can also secrete urease with similar functions.

Urease catalyzes the conversion of urea to carbon dioxide (CO_2) and ammonia (NH_3). The generated ammonia reacts with hypochlorite (OCl^-) to form monochloramine, which further reacts with phenol to form a blue-colored indophenol. Indophenol has a maximum absorption peak at around 650 nm, and within a certain range, the amount of indophenol produced is directly proportional to the urease activity in the sample.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	55 mL × 1 vial	-20°C, 12 months
Reagent 2	Buffer Solution	8 mL × 1 vial	-20°C, 12 months
Reagent 3	Substrate	0.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent A	5.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent B	5.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	Chromogenic Agent C	5.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 7	200 mM NH ₄ Cl Standard Solution	0.2 mL × 1 vial	-20°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Microplate reader (640-660 nm, optimum wavelength: 650 nm), Incubator

Reagent preparation

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

② Substrate Working Solution preparation:

Before testing, please prepare sufficient Substrate Working Solution according to the test wells. For example, prepare 1100 µL of Substrate Working Solution (mix well 1000 µL of Buffer Solution and 100 µL of Substrate). The unused Substrate Working Solution can be stored at -20°C protected from light for 7 days.

③ 2 mmol/L Standard Solution preparation:

Before testing, prepare a sufficient 2 mmol/L Standard Solution according to the test wells. For example, prepare 1000 µL of 2 mmol/L Standard Solution (mix 990 µL of Extraction Solution and 10 µL of 200 mM NH₄Cl Standard Solution thoroughly). Stable for 3 days when stored at 2-8°C.

④ Standard curve preparation:

Always prepare a fresh set of Standards. Discard Working Standard Dilutions after use.

Dilute 2 mmol/L Standard Solution with distilled or deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	0	0.2	0.4	0.6	0.8	1.2	1.6	2
2 mmol/L Standard (µL)	0	20	40	60	80	120	160	200
Extraction Solution (µL)	200	180	160	140	120	80	40	0

Sample preparation

Serum and plasma: detect directly.

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 μ L Buffer Solution with a dounce homogenizer at 4°C.
- ④ Centrifuge at 10000 $\times$ g for 10 min at 4°C. Collect supernatant and keep it on ice for detection and detect within 8 h.
- ⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M).

Microorganism sample:

- ① Harvest the number of microorganism needed for each assay (initial recommendation 1×10^6 cells). Wash microorganism with PBS (0.01 M, pH 7.4).
- ② Homogenize 1×10^6 microorganism in 200 μ L Extraction Solution with a dounce homogenizer.
- ③ Centrifuge at 12000 $\times$ g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection and detect within 8 h.

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Jack bean tissue homogenization	1000-5000
10% Mung bean tissue homogenization	1
10% Pumpkin seed tissue homogenization	1
10% Peanut kernel tissue homogenization	1
10% Edamame bean tissue homogenization	1000-5000
10% Red kidney bean tissue homogenization	1
10% Pine nut kernel tissue homogenization	1

Note: The diluent is Extraction 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 Standard Solution with different concentrations to the corresponding wells.

Blank control well: Add 10 μL of Extraction Solution to the corresponding wells.

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

- ② Add 100 μL of Buffer Solution to standard well and control well.
- ③ Add 100 μL of Substrate Working Solution to Blank control well and sample well.
- ④ Shake the microplate for 5 seconds and incubate at 37°C for 30 min.
- ⑤ Add 50 μL of Chromogenic Agent A to each well.
- ⑥ Add 50 μL of Chromogenic Agent B to each well.
- ⑦ Add 50 μL of Chromogenic Agent C to each well.
- ⑧ Shake the microplate for 5 seconds to ensure complete mixing. Incubate at 37°C for 15 min and measure the OD value of each well at 650 nm.

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 corrected OD value.
3. Plot the standard curve by using corrected 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:

1. Tissue sample:

Calculate according to the weight of sample:

Definition: One unit of enzyme is defined as the amount enzyme in 1 g wet weight will catalyze substrate to produce 1 μmol product per 1 min at 37 °C.

$$\text{urease activity (U/g wet weight)} = (\Delta A - b) \div a \div T \times V \div m \times f \times 1000$$

Calculate according to the protein concentration:

Definition: One unit of enzyme is defined as the amount enzyme in 1 g protein will catalyze substrate to produce 1 μmol product per 1 min at 37 °C.

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

2. Microorganism sample:

Definition: One unit of enzyme is defined as the amount enzyme in 1×10^6 microorganism will catalyze substrate to produce 1 μmol product per 1 min at 37 °C.

$$\text{Urease activity (U/10}^6) = (\Delta A - b) \div a \div T \times V \div n \times f \times 1000$$

[Note]

$\Delta A: (OD_{\text{sample}} - OD_{\text{control}}) - (OD_{\text{blank control}} - OD_{\text{blank}})$.

T: Reaction time, 30min.

m: The weight of tissue, g.

V: The volume of Extraction Solution added to sample homogenization, L

n: The number of cell sample/ 10^6

1000: 1 mmol = 1000 μmol

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

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three edamame bean 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	30	60
%CV	0.7	1.4	0.9

Inter-assay Precision

Three potato 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	30	60
%CV	1.0	1.2	2.0

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 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	10	30	60
Observed Conc. (U/L)	10	29.4	58.2
Recovery rate (%)	100	98	97

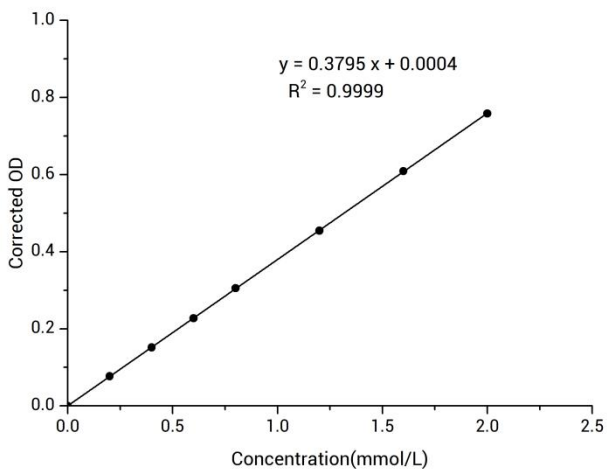
Sensitivity

The analytical sensitivity of the assay is 0.13 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	0.4	0.6	0.8	1.2	1.6	2.0
OD	0.054	0.132	0.207	0.285	0.360	0.512	0.668	0.815
	0.054	0.130	0.204	0.278	0.359	0.505	0.658	0.810
Average OD	0.054	0.131	0.206	0.282	0.360	0.509	0.663	0.813
Corrected OD	0.000	0.077	0.152	0.228	0.306	0.455	0.609	0.759



Appendix Π Example Analysis

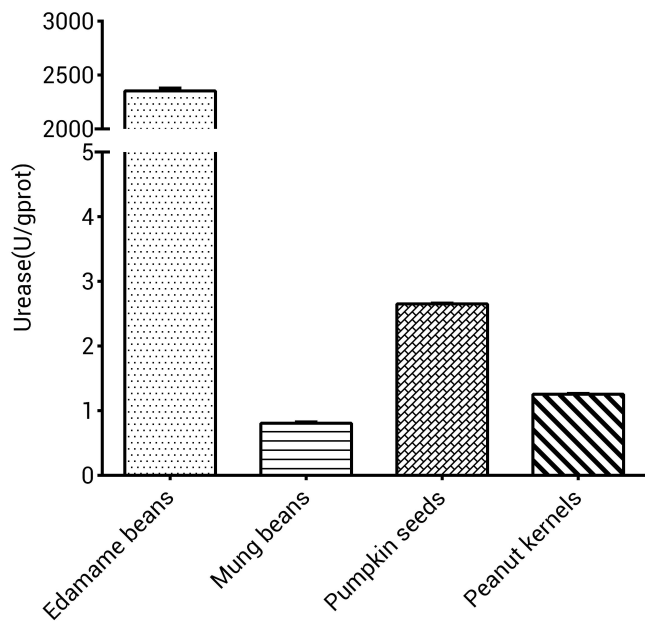
Example analysis :

Take 10 μ L of the 1000-fold diluted 10% edamame bean tissue homogenate to the well of microplate. Proceed according to the operating steps. The results are as follows:

standard curve: $y = 0.4006x - 0.0086$, the value of the sample well is 0.419, the value of the control well is 0.060, the value of the background well is 0.104, the value of the blank well is 0.061, $\Delta A = (0.419 - 0.060) - (0.104 - 0.061) = 0.316$, and the calculation result is:

$$\begin{aligned}\text{Urease activity (U/gprot)} &= (0.316 + 0.0086) \div 0.4006 \div 30 \times 1000 \div 11.51 \times 1000 \\ &= 2346.61 \text{ U/gprot}\end{aligned}$$

Detect 10% edamame beans tissue homogenate supernatant (the concentration of protein is 11.51 gprot/L, dilute for 1000 times), 10% mung beans tissue homogenate supernatant (the concentration of protein is 19.13 gprot/L), 10% pumpkins seed tissue homogenate supernatant (the concentration of protein is 6.70 gprot/L) and 10% peanut kernels tissue homogenate supernatant (the concentration of protein is 21.72 gprot/L), 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.