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

Catalog No：E-BC-K1217-M

Specification：48T(16 samples)/96T(40samples)

Measuring instrument：Microplate reader（500-510 nm）

Detection range：0.92 - 300 U/g

Elabscience® Cellulase (CL) 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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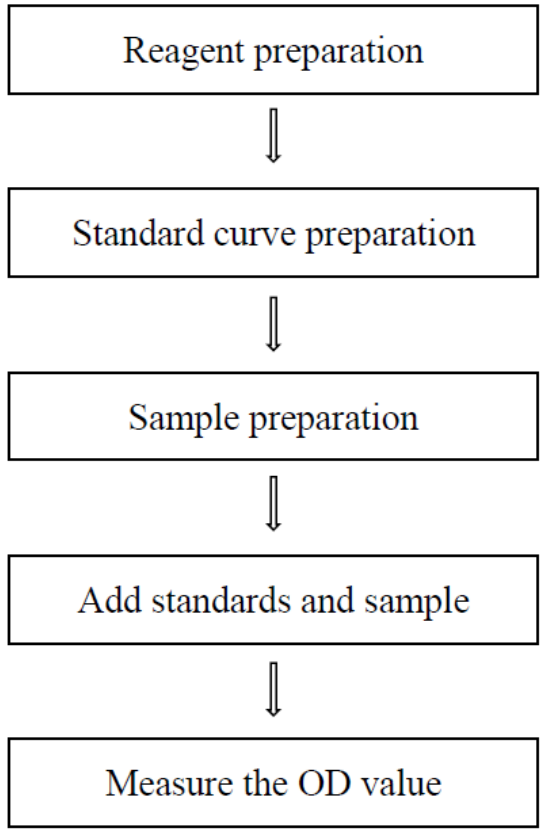
Assay summary



Intended use

This kit can be used to measure the cellulase (CL) activity in soil, tissue, bacteria，bacterial supernatant and other liquid samples.

Detection principle

Cellulase (CL) decomposed the substrate to produce raw sugar. Under the action of the enzyme, it formed colored substances with the chromogen. The activity of CL was measured by the absorbance at 505 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Substrate	10 mL ×1 vial	20 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 2	Phenol Solution	6 mL ×1 vial	12 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 3	Enzyme Solution	6 mL ×1 vial	12 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 4	50 mmol/L Glucose Standard	1.2 mL ×1 vial	1.2 mL ×1 vial	2-8℃, 12 months
Reagent 5	Extracting Solution	50 mL ×1 vial	50 mL ×2 vials	2-8℃, 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 (500 - 510 nm, optimum wavelength 505 nm).

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of enzyme working solution：

For each well, prepare 200 μL of enzyme working solution (mix well 100 μL of phenol solution and 100 μL of enzyme solution). The enzyme working solution should be prepared on spot and stored at 2-8℃ for 24 h protected from light.

③ The preparation of 2 mmol/L standard solution：

Take 40 μL of 50 mmol/L glucose standard and 960 μL of ultrapure water, mix well. The 2 mmol/L standard solution should be stored at 2-8℃ for 24 h protected from light.

④ The preparation of standard curve：

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

Dilute 2 mmol/L standard solution with ultrapure water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.25**	**0.5**	**0.75**	**1**	**1.25**	**1.5**	**2**
2 mmol/L standard (μL)	0	25	50	75	100	125	150	200
Ultrapure water (μL)	200	175	150	125	100	75	50	0

Sample preparation

① Sample preparation

Bacterial supernatant or industrial enzyme: detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 0.1 g).

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

③ Homogenize 0.1 g tissue in 0.9 mL extracting solution with a dounce homogenizer at 4℃.

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

⑤ Determine the protein concentration of supernatant (E-BC-K318-M).

Bacteria sample:

① Harvest the number of bacteria needed for each assay (initial recommendation 5×10^4 bacteria).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 5×10^4 bacteria in 0.9 mL extracting solution with a dounce homogenizer at 4℃ (200 W, ultrasonic 3 s, 10 s interval, 30 times).

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

⑤ Determine the protein concentration of supernatant (E-BC-K318-M).

Soil sample

① Fresh soil sample natural air drying or air dry in oven at 37℃, 30~50 mesh sieve.

② Homogenize 0.1 g soil sample in 0.9 mL 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.

The preparation of control sample: Take a part of the treated sample for enzyme inactivation treatment, boil it in boiling water for 15 min, and then cool it (cold water flow can accelerate cooling) to obtain control sample.

② Dilution of sample

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

Sample type	Dilution factor
10% Soil sample	1
10% Decaying plant tissue homogenate	1-10
Bacteria supernatant	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

① Substrate is slightly sticky, please draw slowly to ensure accurate volume.

② Bath volume losses may be caused in the process, please ensure that the sealing is good.

③ Control samples need to be boiled, please calculate the amount of sample before the experiment.

④ If there is liquid loss during the determination and care of the boiling water bath, please add ultra-pure water to 200μL.

⑤ Substances may be precipitated during the boiling process, centrifuge at 12000×g for 10 min and collect supernatant for detection.

Operating steps

① Sample tube: Add 160 μL of substrate and 40 μL sample into a 0.5 mL EP tube.

Control tube: Add 160 μL of substrate and 40 μL control sample (boiled sample to be tested) into a 0.5 mL EP tube.

② Mix fully, incubate the tubes at 50℃ with water bath for 1 h. After incubation, put the EP tube into boiling water and boiled for 15 min, the saccharifying liquid was obtained after cooling, and the saccharifying liquid was added into the well.

③ Sample well: Add 50 μL of saccharifying liquid in the sample tube into wells.

Control well: Add 50 μL of saccharifying liquid in the control tube into wells.

Standard well: Add 50 μL of standard solution with different concentrations into wells.

④ Take 200 μL of the enzyme working solution to each well, incubate at 37℃ for 15 min and measure the OD values of each well at 505 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:

1. Liquid sample:

Definition: The amount of enzyme in 1mL liquid sample per 1 min that produce 1 μg glucose at 50 ℃ is defined as 1 unit.

$$\text{cellulase activity (U/mL)} = \frac{(\Delta A - b) \div a \times V_3 \times f \times 180.16^{*}}{T \times V_2 \div V_1} = (\Delta A - b) \div a \times f \times 15.01$$

2. Tissue sample (Calculated in tissue quality):

Definition: The amount of enzyme in 1g sample per 1 min that produce 1 μg glucose at 50 ℃ is defined as 1 unit.

$$\text{cellulase activity (U/g)} = \frac{(\Delta A - b) \div a \times V_3 \times f \times 180.16^{*}}{T \times m \times V_2 \div V_1} = (\Delta A - b) \div a \div m \times f \times 15.01$$

3. Tissue sample (Calculated in protein concentration):

Definition: The amount of enzyme in 1mg protein per 1 min that produce 1 μg glucose at 50 ℃ is defined as 1 unit.

$$\text{cellulase activity (U/gprot)} = \frac{(\Delta A - b) \div a \times V_3 \times f \times 180.16^{*}}{T \times C_{pr} \times V_2} = (\Delta A - b) \div a \div C_{pr} \times f \times 15.01$$

4. Bacteria sample:

Definition: The amount of enzyme in 10^4 cell per 1 min that produce 1 μg glucose at 50 ℃ is defined as 1 unit.

$$\text{cellulase activity } (U/10^4) = \frac{(\Delta A - b) \div a \times V_3 \times f \times 180.16^*}{T \times N \times V_2 \div V_1} = (\Delta A - b) \div a \div N \times f \times 15.01$$

[Note]

ΔA: $OD_{Sample} - OD_{Contrast}$.

V1: The volume of sample, mL.

V2: The volume of sample added to the reaction system, mL.

V3: The volume of reaction system, mL.

m: Weigh the quality of the sample, g.

C_{pr}: Concentration of protein in sample, mg/mL.

N: The number of bacteria sample/10^4.

f: The dilution multiple of tested samples.

T: Enzymatic reaction time, 60 min.

180.16: Micromolar mass of glucose, μg/μmol.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three soil 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/g）	75	150	250
%CV	4.9	4.2	3.5

Inter-assay Precision

Three soil samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/g)	75	150	250
%CV	5.3	4.5	4.8

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 99.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/g)	75	150	250
Observed Conc. (U/g)	76.2	145.6	247.2
recovery rate(%)	102	97	99

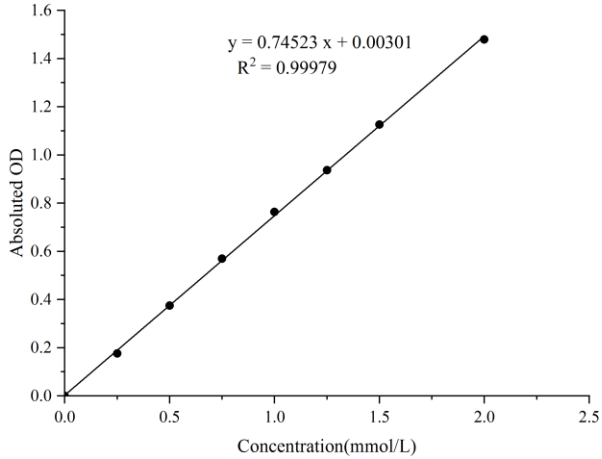
Sensitivity

The analytical sensitivity of the assay is 0.92 U/g. 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**	**0.25**	**0.5**	**0.75**	**1**	**1.25**	**1.5**	**2**
OD value	0.043	0.218	0.418	0.607	0.814	0.984	1.178	1.529
	0.043	0.22	0.418	0.619	0.8	0.975	1.16	1.515
Average OD	0.043	0.219	0.418	0.613	0.807	0.980	1.169	1.522
Absoluted OD	0.000	0.176	0.375	0.570	0.764	0.937	1.126	1.479



Appendix II Example Analysis

Example analysis：

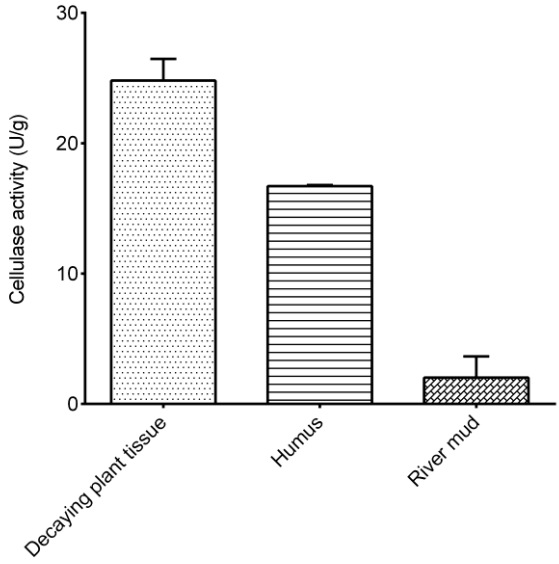
Take 200 μL of 10% decaying plant tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.74523x + 0.00301, the average OD value of the sample is 0.240, the average OD value of the contrast is 0.115, calculated by sample quality and the calculation result is:

$$\text{cellulase activity (U/g)} = (0.240 - 0.115 - 0.00301) \div 0.74523 \div 0.1 \times 1 \times 15.01$$

$$= 24.57 \text{ U/g}$$

Detect 10% decaying plant tissue homogenate (dilute for 10 times), 10% Humus sample (dilute for 10 times)and 10% river mud sample (dilute for 10 times), 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.