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

Catalog No：E-BC-K630-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(395-415 nm)

Detection range：0.02-29.06 U/L

Elabscience®α-Galactosidase (α-Gal) Acitivity 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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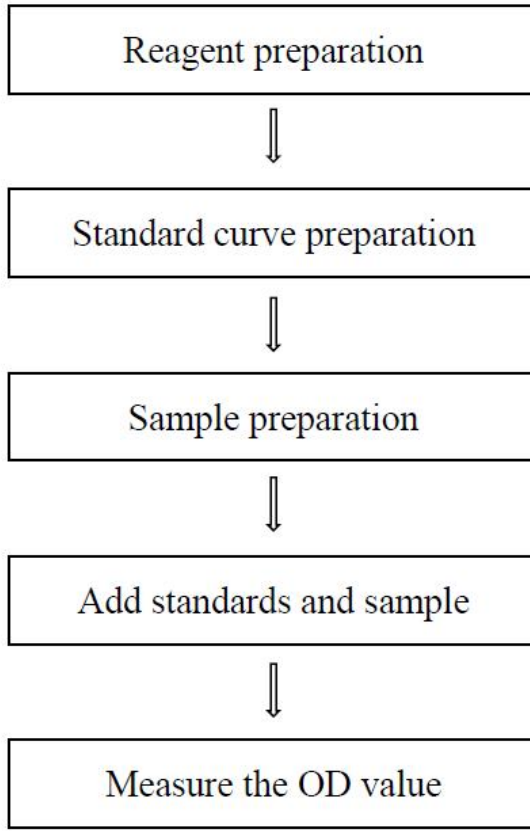
Assay summary



Intended use

This kit can be used to measure the α-Galactosidase (α-Gal) activity in animal and plant tissue samples.

Detection principle

α-Galactosidase (α-Gal) catalyzes the substances produced in the glycolysis process of animal and plant tissues to form the final product, which has a maximum absorption peak at 405 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 2 vials	-20℃, 12 months
Reagent 2	Substrate	Powder × 1 vial	-20℃, 12 months shading light
Reagent 3	Accelerant	1.6 mL × 1 vial	-20℃, 12 months shading light
Reagent 4	Chromogenic Agent	13 mL × 1 vial	-20℃, 12 months
Reagent 5	10 mmol/L Standard Solution	0.3 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 (395-415 nm, optimum wavelength: 405 nm) Vortex mixer, Centrifuge

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate working solution：
Dissolve one vial of substrate with 1.3 mL of double distilled water, mix well. Store at 2-8°C for 4 weeks.

③ The preparation of measuring working solution：
For each well, prepare 135 uL of measuring working solution (mix well 100 uL of buffer solution, 20 uL of substrate working solution and 15 uL of accelerant). Store at 2-8°C for 1 month protected from light.

④ The preparation of control working solution：
For each well, prepare 135 uL of control working solution (mix well 120 uL of buffer solution and 15 uL of accelerant). Store at 2-8°C for a month protected from light.

⑤ The preparation of 0.5 mmol/L standard solution：
Dilute 0.05 mL of 10 mmol/L standard solution with 0.95 mL of double distilled water, and keep it on ice protected from light for detection. The 0.5 mmol/L standard solution should be prepared on spot and use up within 8 h.

⑥ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 0.5 mmol/L standard with double distilled water to a serial

concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.15, 0.2, 0.3, 0.4, 0.45, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.15**	**0.2**	**0.3**	**0.4**	**0.45**	**0.5**
0.5 mmol/L standard (mL)	0	40	60	80	120	160	180	200
Double distilled water (mL)	200	160	140	120	80	40	20	0

Sample preparation

① Sample preparation

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 μL buffer solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes 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
10% Sweet potato tissue homogenate	1
10% Wood mushroom tissue homogenate	1
10% Flammulina velutipes tissue homogenate	1
10% Mouse liver 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

① Standard well: add 10 μL of standard solution with different concentration to the corresponding well.

Sample well: add 10 μL of sample to the corresponding well.

Control well: add 10 μL of sample to the corresponding well.

② Add 135 μL of measuring working solution to the sample wells and standard wells.

Add 135 μL of control working solution to the control wells.

③ Mix well with microplate reader for 5 s and incubate at 37℃ for 30 min. Add 100 μL of chromogenic agent to each well.

④ Mix well with microplate reader for 5 s, and measure the OD value of each well at 405 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 α-Gal in 1 g sample protein that hydrolyze the substrate to produce 1 μmol product in 1 minute at 37°C is defined as 1 unit.

$$\underset{\text{(U/gprot)}}{\text{α-Gal activity}} = (\Delta A_{405} - b) \div a \div T \times f \div C_{pr} \times 1000$$

[Note]

ΔA_{405}: $OD_{Sample} - OD_{control}$

f: Dilution factor of sample before test.

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

T: Reaction time, 30 min.

1000: 1 mmol/L=1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three sweet potato 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/L)	0.50	16.00	27.00
%CV	3.0	2.6	1.0

Inter-assay Precision

Three sweet potato 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/L)	0.50	16.00	27.00
%CV	8.0	6.9	4.0

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 99%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.13	0.25	0.47
Observed Conc. (mmol/L)	0.1	0.2	0.5
Recovery rate (%)	98	99	100

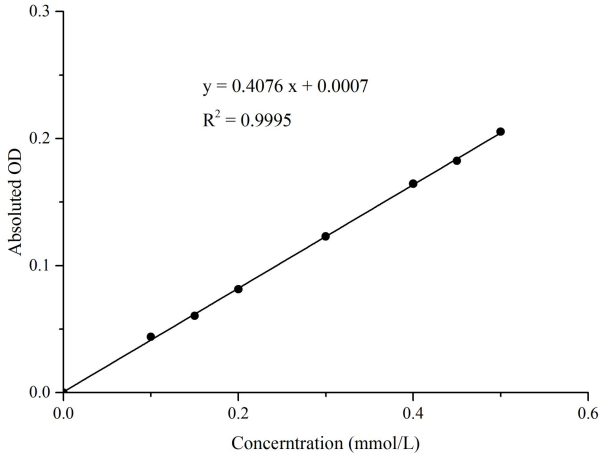
Sensitivity

The analytical sensitivity of the assay is 0.02 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**	**0.1**	**0.15**	**0.2**	**0.3**	**0.4**	**0.45**	**0.5**
OD value	0.1	0.144	0.161	0.181	0.221	0.264	0.281	0.305
	0.1	0.143	0.159	0.181	0.224	0.264	0.283	0.305
Average OD	0.1	0.144	0.160	0.181	0.223	0.264	0.282	0.305
Absoluted OD	0	0.044	0.061	0.081	0.123	0.164	0.182	0.205



Appendix II Example Analysis

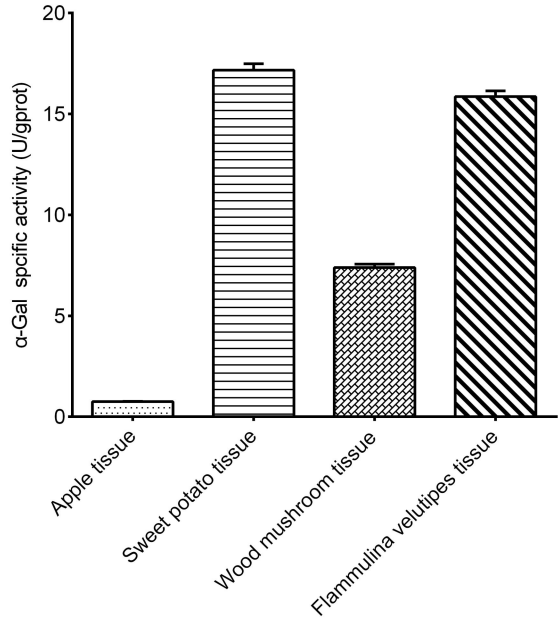
Example analysis：

Take 10 μL of 10% apple tissue supernatant sample carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.4076 x + 0.0007, the average OD value of the control is 0.078, the average OD value of the sample is 0.130, the concentration of protein in sample is 5.63 gprot/L, and the calculation result is:

α-Gal activity(U/gprot) = (0.078 - 0.130 - 0.0007) ÷ 0.4076 ÷ 30 ÷ 5.630 × 100 = 0.75 U/gprot

Detect 10% apple tissue homogenate (the concentration of protein is 5.63 gprot/L), 10% sweet potato tissue homogenate (the concentration of protein is 3.60 gprot/L), 10% wood mushroom tissue homogenate (the concentration of protein is 3.98 gprot/L) and 10% flammulina velutipes tissue homogenate (the concentration of protein is 2.23 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.