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

Catalog No：E-BC-K631-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（390-410 nm）

Detection range：16.19-1200.0 U/L

Elabscience®β - Galactosidase (β –GAL)

Activity 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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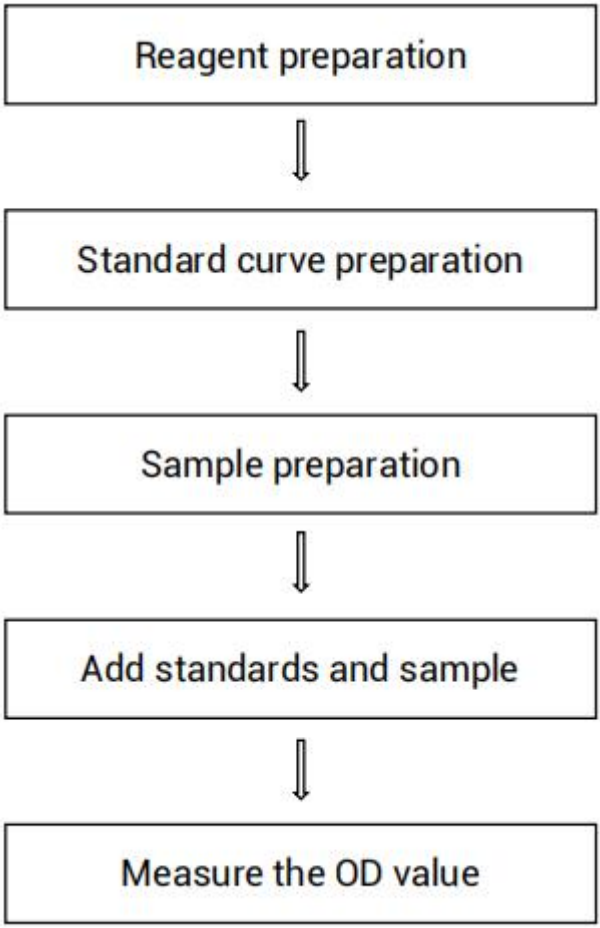
Assay summary



Intended use

This kit can measure β-galactosidase activity in cells, animal and plant tissue samples.

Detection principle

β-galactosidase (β-GAL) can catalyze the hydrolysis of lactose and glycoside conversion., widely involved in the growth and development of plants and the synthesis of plant cell walls. β-galactosidase can hydrolyze lactose from milk and whey to produce galactose and glucose, and can also be used to treat lactose intolerance caused by lactose deficiency in human body. In addition, the study of β-galactosidase plays an important role in the field of biotechnology, such as gene engineering, enzyme engineering and protein engineering.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Substrate	Powder × 1 vial	-20℃, 12 months
Reagent 3	Activator Agent	1.5 mL × 1 vial	2-8℃, 12 months shading light
Reagent 4	Chromogenic Agent	25 mL×1 vial	2-8℃, 12 months
Reagent 5	20 mmol/L Standard Solution	1 mL × 1 vial	2-8℃, 12 months
	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:

Water bath, Incubator, Centrifuge, Microplate reader (390-410 nm, optimum wavelength: 400 nm)

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of substrate working solution：
Dissolve one vial of Substrate with 2 mL of buffer solution in 90-100°C water bath, cool to room temperature before use. Store at 2-8°C for 7 days protected from light.

③ The preparation of 1 mmol/L standard solution：
Dilute 40 μL of 20 mmol/L standard solution with 760 μL of double distilled water, mix well. Store at 2-8°C for 7 days.

④ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0.0, 0.1, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**
1 mmol/L standard (μL)	0	20	40	80	100	120	140	160
Double distilled water (μL)	200	180	160	120	100	80	60	40

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 min at 4℃ 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).

Cell (adherent or suspension) samples:

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

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

③ Homogenize 1×10^6 cells in 200 μL buffer solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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% Mouse liver tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat kidney tissue homogenate	3-5
10% Rat liver tissue homogenate	1-3
10% Rat heart tissue homogenate	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

① To avoid contamination, it is recommended to aliquot the 20 mmol/L standard solution into smaller quantities before use.

② Preheat substrate working solution to dissolve it, and cool to room temperature before use.

Operating steps

① Standard well: Take 20 μL of standard solution with different concentrations to the corresponding wells.
Sample well: Take 20 μL of sample to the corresponding wells.
Control well: Take 20 μL of sample to the corresponding wells.
② Add 30 μL of substrate working solution to the sample wells, and add 30 μL of buffer solution to the control wells and standard wells.
③ Add 10 μL of activator agent to each well.
④ Mix fully with microplate reader and incubate at 37℃ for 40 min.
⑤ Add 140 μL of chromogenic agent to each well.
⑥ Mix fully with microplate reader and incubate at 37℃ for 10 min with shading light.
⑦ Measure the OD value of each well at 400 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. Tissue and cells sample (Calculated by tissue protein):

Definition: The amount of β-GAL in 1 g tissue protein per 1 h that hydrolyze the substrate to produce 1 μmol p-nitrophenol at 37°C is defined as 1 unit.

$$\text{β-GAL activity (U/gprot)} = (\Delta A_{400} - b) \div a \div C_{pr} \div T \times f \times 1000*$$

2. Tissue sample (Calculated by tissue wet weight):

Definition: The amount of β-GAL in 1 kg tissue per 1 h that hydrolyze the substrate to produce 1 μmol p-nitrophenol at 37°C is defined as 1 unit.

$$\text{β-GAL activity (U/kg/wet weight)} = (\Delta A_{400} - b) \div a \div (m \div V) \div T \times f \times 1000*$$

[Note]

ΔA_{400}: $OD_{Sample} - OD_{Control}$.

T: The time of incubation reaction, 2/3 h.

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

V: The volume of reagent 1, 0.9 mL.

m: The weight of the sample,0.1 g.

f: Dilution factor of sample before test.

1000*: 1 mmol/L =1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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)	25.60	435.00	653.00
%CV	3.2	3.0	2.8

Inter-assay Precision

Three human serum 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)	25.60	435.00	653.00
%CV	5.6	6.3	6.1

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 101%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.15	0.46	0.77
Observed Conc. (mmol/L)	0.2	0.5	0.8
Recovery rate (%)	101	99	103

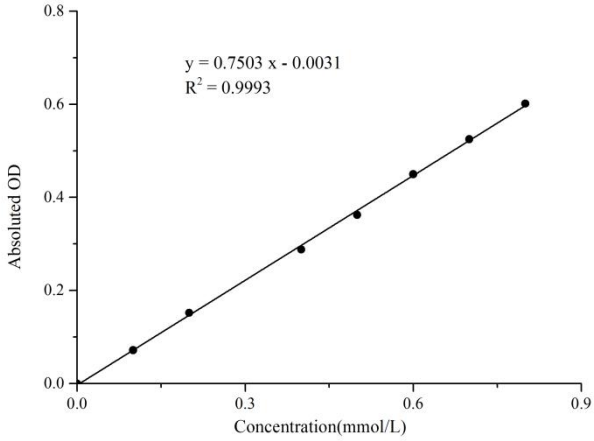
Sensitivity

The analytical sensitivity of the assay is 16.19 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	0.2	0.4	0.5	0.6	0.7	0.8
Average OD	0.040	0.112	0.192	0.328	0.403	0.490	0.565	0.640
Absoluted OD	0.000	0.072	0.152	0.288	0.363	0.450	0.525	0.600



Appendix Π Example Analysis

Example analysis：

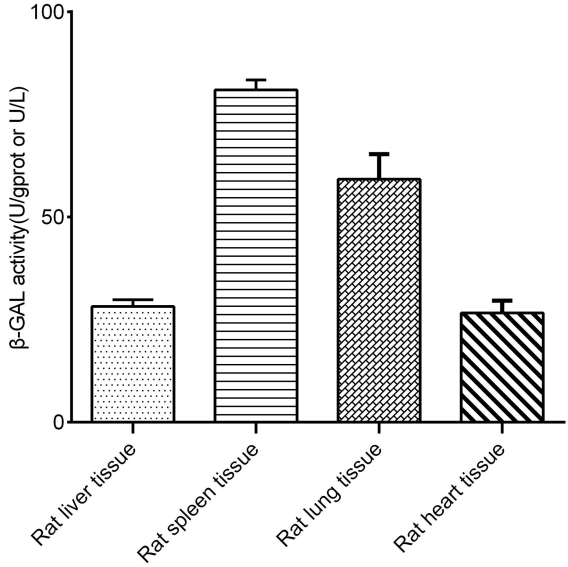
For 10% rat liver tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.7503x - 0.0031, the average OD value of the control is 0.190, the average OD value of the sample is 0.364, the concentration of protein in sample is 12.50 gprot/L, and the calculation result is:

β-GAL activity (U/gprot) = (0.364 - 0.190 + 0.0031) ÷ 0.7503 ÷ 12.5÷ 2/3 × 1000

= 28.32 U/gprot

Detect 10% rat liver tissue homogenate (the concentration of protein is 12.50 gprot/L), 10% rat spleen tissue homogenate (the concentration of protein is 7.51 gprot/L), 10% rat lung tissue homogenate (the concentration of protein is 3.05 gprot/L) and 10% rat heart tissue homogenate (the concentration of protein is 4.41 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.