PRODUCT INFORMATION & MANUAL

beta Galactosidase Activity Assay Kit (Colorimetric)

NBP3-24551

For research use only.
Not for diagnostic or therapeutic procedures.

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beta Galactosidase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24551

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 16.19 U/L

Detection range: 16.19-1200.0 U/L

Average intra-assay CV (%): 3.0

Average inter-assay CV (%): 6.0

Verage recovery rate (%): 101

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲Background

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.

▲Detection principle

β-galactosidase can catalyze the substrate nitrophenylgalactoside pyranopyranoside to produce nitrophenol, which has a maximum absorption peak at 400 nm. The activity of β-GAL can be calculated by measuring the OD value at 400 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 2	Substrate	Powder × 1 vial	-20°C, 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	
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.			

▲Materials prepared by users

Instruments

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

Reagents:

Double distilled water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. To avoid contamination, it is recommended to aliquot the reagent 5 into smaller quantities before use.
2. Preheat reagent 2 working solution to dissolve it, and cool to room temperature before use.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 2 working solution:

   Dissolve the reagent 2 with 2 mL of reagent 1 in 90-100°C water bath, cool to room temperature before use. The prepared solution can be stored at 2-8°C for 7 days with shading light.
3. Preparation of 1 mmol/L standard solution:

   Dilute reagent 5 with double distilled water at a ratio of 1:19. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°C for 7 days.

▲ Sample preparation

1. Serum and plasma samples:

   Detect the sample directly. If the sample is turbidity, centrifuge at 10000 g for 10 min, then take the supernatant for detection.
2. Tissue sample:

   Accurately weigh the tissue, add 9 times the volume of reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min. If the supernatant is turbidity after centrifugation, centrifuge the supernatant repeatedly before use. Meanwhile, determine the protein concentration of supernatant.
3. Cell sample:

   Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): reagent 1 (μL) =1: 200. Sonicate or mechanical homogenate in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (16.19-1200.0 U/L).

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
Human serum	1

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note:A-H, standard wells; S1-S40, control wells; S1'-S40', sample wells.

▲Detailed operating steps

1. The preparation of standard curve

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:

Number	Standard concentrations (mmol/L)	1 mmol/L Standard(μL)	Double distilled water (μL)
A	0	0	200
B	0.1	20	180
C	0.2	40	160
D	0.4	80	120
E	0.5	100	100
F	0.6	120	80
G	0.7	140	60
H	0.8	160	40

2. The measurement of samples

(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 regent 2 working solution to the sample wells, and add 30 μL of reagent 1 to the control wells and standard wells.

(3) Add 10 μL of reagent 3 to each well.

(4) Mix fully with microplate reader and incubate at 37℃ for 40 min.

(5) Add 140 μL of reagent 4 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.

▲Summary operation table

	Standard well	Control well	Sample well
Standard solution with different concentrations (μL)	20		
Sample (μL)		20	20
Reagent 1 (μL)	30	30	
Reagent 2 working solution (μL)			30
Reagent 3 (μL)	10	10	10
Mix fully and incubate at 37℃ for 40 min.			
Reagent 4 (μL)	140	140	140
Mix fully and incubate at 37℃ for 10 min with shading light. Measure the OD value at 400 nm.			

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

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.

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

3. Serum/plasma sample:

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

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

Note:

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

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

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

C_{pr}: 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 tested.

1000*: 1 mmol/L =1000 μmol/L.

Appendix I Data

▲Example analysis

For 10% rat liver tissue homogenate, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.7503 x - 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