PRODUCT INFORMATION & MANUAL

Lactase Activity Assay Kit (Colorimetric)

NBP3-25846

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

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Lactase Activity Assay Kit

Catalog No: NBP3-25846

Method: Colorimetric method

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

Measuring instrument: Microplate reader

Sensitivity: 3.94 U/mL

Detection range: 12.5-2000 U/mL

Average intra-assay CV (%): 4.5

Average inter-assay CV (%): 8.5

Average recovery rate (%): 102

- ▲ 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 lactase activity in animal tissue samples.

▲Background

Lactase is a glycoprotein with two active sites, which can catalyze the hydrolysis of various β-glycosidic bonds. It can catalyze the decomposition of lactose into glucose and galactose. Lactase plays a vital role in the nutrition of newborns in humans and other mammals, because this enzyme is the only small intestinal brush border hydrolase responsible for digesting lactose. Low levels of lactase and inability to digest lactose may cause lactose intolerance.

▲Detection principle

Lactase decomposes lactose to produce glucose. Under the action of enzyme, glucose produces hydrogen peroxide. In the presence of chromogenic oxygen receptors, peroxidase catalyzes hydrogen peroxide to produce colored substances. Lactase activity can be calculated by measuring the OD value at 505 nm.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Substrate	Powder × 1 vial	2-8℃ , 12 months
Reagent 2	Buffer Solution	10 mL x 1 vial	2-8℃ , 12 months
Reagent 3	Stop Solution	6 mL x 1 vial	2-8℃ , 12 months
Reagent 4	Phenol Solution	12 mL x 1 vial	2-8℃ , 12 months, shading light
Reagent 5	Enzyme Solution	12 mL x 1 vial	2-8℃ , 12 months, shading light
Reagent 6	50 mmol/L Glucose Standard Solution	1.5 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

Micropipettor, Vortex mixer, Centrifuge, Water bath, Incubator, Microplate reader (505 nm)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

⚠ 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. The temperature and time of incubation at 37℃ must be accurately.
2. If the lactase activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M).
3. Accurate operation is required when adding liquid to microplate and prevent the formulation of bubbles when adding the liquid to the microplate.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 1 working solution:
   Dissolve a vial of reagent 1 with 8 mL of reagent 2 and mix fully. The prepared solution can be stored at 2-8℃ for a month.
2. Preparation of chromogenic agent:
   Mix the reagent 4 and reagent 5 fully at the ratio of 1:1. Prepare the needed fresh solution before use.

▲Sample preparation

Tissue sample: Weigh the tissue accurately and add normal saline at a ratio of weight (g): volume (mL) =1: 9, homogenize the tissue in ice bath, centrifuge at 10000 g for 10 min at 4℃ , then take the supernatant for measurement.

▲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 (12.5-2000 U/mL).

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

Sample type	Dilution factor
10% Rat Ileal tissue homogenate	1
10% Rat jejunum tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1

[Note]: The diluent is normal saline (0.9% NaCl).

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, sample wells; S1'– S40', control wells.

▲Detailed operation steps

The preparation of standard curve

Dilute 50 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 2, 5, 10, 15, 20, 30, 40 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	50 mmol/L standard solution (μL)	Double distilled water (μL)
A	0	0	100
B	2	4	96
C	5	10	90
D	10	20	80
E	15	30	70
F	20	40	60
G	30	60	40
H	40	80	20

2. The measurement of samples

1) Standard tube: add 25 μL of standard solution with different concentrations to the corresponding 1.5 mL EP tubes.
   Sample tube: add 25 μL of sample to the corresponding 1.5 mL EP tubes.
   Control tube: add nothing.
2) Add 50 μL of reagent 1 working solution to each tube.
3) Mix fully and react at 37℃ for 20 min.
4) Add 25 μL of reagent 3 to each tube.

5) Standard tube: add nothing.

Sample tube: add nothing.

Control tube: add 25 μL of sample to the corresponding 1.5 mL EP tubes.

6) Mix fully and centrifuge at 1780 g for 10 min.

7) Take 8 μL of the supernatant to corresponding wells in microplate.

8) Add 200 μL of chromogenic agent to each well.

9) Mix fully for 10 s with microplate reader, incubate at 37℃ for 15 min and measure the OD value of each well at 505 nm.

▲Summary operation table

	Standard	Sample	Control
Standard solution with different concentrations (μL)	25		
Sample (μL)		25	
Reagent 1 working solution (μL)	50	50	50
Mix fully and react at 37℃ for 20 min.			
Reagent 3 (μL)	25	25	25
Sample (μL)			25
Mix fully and centrifuge at 1780 g for 10 min, take supernatant to corresponding wells in microplate.			
Supernatant (μL)	8	8	8
Chromogenic agent (μL)	200	200	200
Mix fully, incubate at 37℃ for 15 min and measure the OD value of each well.			

▲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.

$$\text{Lactase activity (U/mgprot)} = (\Delta A - b) \div a \div 20^{*} \times 1000^{**} \times f \div C_{pr}$$

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.

f: Dilution factor of sample before tested.

ΔA: $OD_{Sample} - OD_{Control.}$

20*: Reaction time, 20 min

1000**: 1 μmol=1000 nmol.

C_{pr}: The concentration of protein in sample, mgprot/mL.

Appendix I Data

▲Example analysis

For rat lleal tissue, take 25 μL of 10% rat lleal tissue homogenate and carry the assay according to the operation table. The results are as follows:

standard curve: y =0.0342 x - 0.0078, the average OD value of the sample is 0.065, the average OD value of the blank is 0.053, the concentration of protein in sample is 5.19 mgprot/mL, and the calculation result is:

Lactase activity (U/mgprot) = (0.065 – 0.053 + 0.0078) ÷ 0.0342 ÷ 20 ×1000 ÷ 5.19 = 5.58 U/mgprot