PRODUCT INFORMATION & MANUAL

Maltase Activity Assay Kit (Colorimetric)

NBP3-25899

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

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Maltase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25899

Method: Colorimetric method

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

Measuring instrument: Microplate reader

Sensitivity: 6.32 U/mL

Detection range: 6.32-750 U/mL

Average intra-assay CV (%): 2.7

Average inter-assay CV (%): 5.7

Average recovery rate (%): 103

- ▲ 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 maltase activity in animal tissue samples.

▲Background

Maltose (EC 3.2.1.20) is a disaccharase that mainly exists in the brush border of epithelial cells and belongs to lysosomal enzymes. Maltase can catalyze the hydrolysis of maltose to the monosaccharide glucose, which also exists in plants, bacteria and yeast.

▲Detection principle

Maltase catalyze the corresponding substrate to produce monosaccharide. Monosaccharide produce hydrogen peroxide under the action of oxidase. Hydrogen peroxide react with chromogenic agent to form red product. The activity of maltase can be calculated by detection the optical density with spectrophotometer at 505 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Substrate	Powder × 1 vial	2-8℃ , 12 months
Reagent 2	Buffer Solution	8 mL x 1 vial	2-8℃ , 12 months
Reagent 3	Stop Solution	3.5 mL x 1 vial	2-8℃ , 12 months
Reagent 4	Chromogenic Agent A	13 mL x 1 vial	2-8℃ , 12 months, shading light
Reagent 5	Chromogenic Agent B	13 mL x 1 vial	2-8℃ , 12 months, shading light
Reagent 6	50 mmol/L Glucose 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

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

Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

⚠ 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. If the OD value of sample tube is more than 1, please dilute the sample and test again.
2. If the lactase activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately.
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 6 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

Sample requirements

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 (6.32-750 U/mL).

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

Sample type	Dilution factor
10% Rat heart tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Mouse intestinal tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

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

1. The preparation of standard curve

Dilute 50 mmol/L glucose standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 2.5, 5, 10, 15, 20, 25, 30 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	50 mmol/L glucose standard (μL)	Double distilled water (μL)
A	0	0	400
B	2.5	20	380
C	5	40	360
D	10	80	320
E	15	120	280
F	20	160	240
G	25	200	200
H	30	240	160

2. The measurement of standard curve

1) Take 1.5 mL EP tube and number the tubes from A to H in duplication, add 25 μL of standard solution with different concentrations to the corresponding tubes.
2) Add 50 μL of reagent 1 working solution to each tube.
3) Add 25 μL of reagent 3 to each tube.
4) Mix fully with a vortex mixer and take 8 μL of supernatant to the corresponding wells in microplate.
5) Add 200 μL of chromogenic agent to each well.
6) Mix fully for 5 s with microplate reader, incubate at 37℃ for 15 min and measure the OD value of each well at 505 nm.

3. The measurement of sample

1) Sample tube: add 25 μL of sample and 50 μL of reagent 1 working solution to the corresponding 1.5 mL EP tubes.

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

   Control tube: add 25 μL of sample to the corresponding 1.5 mL EP tubes.
5) Mix fully with a vortex mixer and centrifuge at 3000 g for 10 min
6) Take 8 μL of the supernatant to corresponding wells in microplate.
7) Add 200 μL of chromogenic agent to each well.
8) Mix fully for 5 s with microplate reader, incubate at 37℃ for 15 min and measure the OD value of each well at 505 nm.

▲Summary operation table

The measurement of standard curve

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

The measurement of sample

	Sample	Control
Sample (μL)	25	
Reagent 1 working solution (μL)	50	50
Mix fully and react at 37℃ for 20 min.		
Reagent 3 (μL)	25	25
Sample (μL)		25
Mix fully and centrifuge at 3000 g for 10 min. Take 8 μL of the supernatant to corresponding wells in microplate.		
Chromogenic agent (μL)	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: $y= ax + b$.

Definition: The amount of 1 nmol of maltose hydrolyzed by 1 mg of tissue protein per minute at 37 ℃ and pH 6.0 is defined as 1 unit.

$$\text{Maltase activity (U/mgprot)} = (\Delta A_{505} - b) \div a \div 2^{*} \div t \div C_{pr} \times 1000^{**}$$

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.

f: Dilution factor of sample before tested.

ΔA_{505}: $OD_{sample} - OD_{control.}$

2^{*}: A maltose molecule can be decomposed into two glucose molecules.

t: Reaction time, 20 min

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

1000^{**}: 1 mmol/L=1000 nmol/mL.

Appendix I Data

▲Example analysis

For mouse intestinal tissue, take 10% fresh mouse intestinal tissue homogenate and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0356 x – 0.0009, the average OD value of the sample is 0.900, the average OD value of the control is 0.162, the concentration of protein in sample is 4.56 mgprot/mL, and the calculation result is:

Maltase activity (U/mgprot)

= (0.900 –0.162+0.0009) ÷ 0.0356 ÷2 ÷ 20 ÷ 4.56 × 1000 = 113.79 U/mgprot