PRODUCT INFORMATION & MANUAL

Lactase Activity Assay Kit (Colorimetric)

NBP3-25845

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

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Lactase Activity Assay Kit

Catalog No: NBP3-25845
Method: Colorimetric method
Specification: 50Assays **(**Can detect 24 samples without duplication)
Measuring instrument: Spectrophotometer, Microplate reader
Sensitivity: 2.0 U/mL
Detection range: 2.0-1600 U/mL

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.

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

This kit can be used for detection of the lactase activity in tissue samples.

Detection significance

Lactase is an enzyme produced by many organisms. It is located in the brush border of the small intestine of humans and other mammals. Lactase is essential to the complete digestion of whole milk; it breaks down lactose, a sugar which gives milk its sweetness. Lacking lactase, a person consuming dairy products may experience the symptoms of lactose intolerance. Lactase can be purchased as a food supplement, and is added to milk to produce "lactose-free" milk products. Lactase (also known as lactase-phlorizin hydrolase, or LPH), a part of the β-galactosidase family of enzymes, is a glycoside hydrolase involved in the hydrolysis of the disaccharide lactose into constituent galactose and glucose monomers. Lactase is present predominantly along the brush border membrane of the differentiated enterocytes lining the villi of the small intestine. In humans, lactase is encoded by the LCT gene.

Detection principle

Lactase react with the corresponding substrate and produce monosaccharide, which produces hydrogen peroxide under the catalyzation of its oxidase. Hydrogen peroxide combines with the chromogenic agent to produce red products. Measure the OD value with spectrophotometer, and the lactase activity can be determined according to the measured OD value.

Kit components

	Components	**Specification**	**Storage**
Reagent 1 (A)	Powder	1 vial	2-8℃, 12 months
Reagent 1 (B)	Diluent	10 mL ×1 vial	2-8℃, 12 months
Preparation of Substrate: dissolve a vial of reagent 1 powder with a vial of reagent 1 diluent fully before use. Store the prepared solution at 2-8℃.			
Reagent 2	Stop Solution	5 mL ×1 vial	2-8℃, 12 months
Note: It will solidify in cold weather. Incubate the solidified reagent 2 with water bath to accelerate the dissolvement until it turns transparent.			
Reagent 3	Liquid	50 mL ×1 vial	2-8℃, 12 months shading light
Reagent 4	5.55 mmol/L Glucose Standard Solution	0.1 mL ×1 vial	2-8℃, 12 months
Preparation of 1.85 mmol/L glucose standard solution: Dilute reagent 4 with double distilled water at a ratio of 1:2. Prepare the fresh needed amount before use.			

Experimental instrument

Tube tube, Micropipette, Vortex mixer, Centrifuge, Spectrophotometer (505 nm) / Microplate reader

(505 nm), 37℃ water bath

The preparation of tissue sample

10% tissue homogenate sample: Accurately weigh the tissue sample, add 9 times the volume of PBS (0.01 M, pH7~7.4) according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 3500 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S).

Operation table

1. Enzymatic reaction

	Sample tube	Control tube
Sample (μL)	25	
Substrate (μL)	50	50
Mix fully and incubate for 20 min at 37℃.		
Reagent 2 (μL)	25	25
Sample (μL)		25
Mix fully and centrifuge at 4000 rpm for 10 min, then take the supernatant for chromogenic reaction.		

2. Chromogenic reaction

1) Measured by microplate reader

	Blank well	Standard well	Sample well	Control well
Double distilled water (μL)	8			
1.85 mmol/L Glucose standard solution (μL)		8		
Supernatant (μL)			8	8
Reagent 3 (μL)	200	200	200	200
Mix fully with microplate reader and incubate at 37℃ for 15 min. Measure the OD value of each well at 505 nm with microplate reader.				

2) Measured by spectrophotometry

	Blank tube	Standard tube	Sample tube	Control tube
Double distilled water (μL)	40			
1.85 mmol/L Glucose standard solution (μL)		40		
Supernatant (μL)			40	40
Reagent 3 (μL)	1000	1000	1000	1000
Mix fully and incubate the tubes at 37℃ for 15 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 505 nm with 1 cm optical path cuvette.				

[Note] The optimum sampling concentration should be determined by pre-experiment before batch experiment, so that the ($OD_{sample} - OD_{control}$) should be closed with $OD_{standard}$.

Calculation of results

1. **Definition:** The amount of lactase in 1 mg of protein tissue that hydrolyze 1 nmol of lactose per minute at 37℃ and pH 6.0 is defined as 1 unit.

2. **Calculation formula:**

$$\text{tivity (U/mgprot)} = \frac{\Delta A_1}{\Delta A_2} \times \quad \times V1 \div t \div (Cpr \times V2) \times 10\text{^}6$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Control}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: The concentration of standard, 1.85 mmol/L.

V_1: The total volume of enzymatic reaction, $0.1*10^{-3}$L.

V_2: The volume of sample, 0.025mL.

t: The enzymatic reaction time, 20 min.

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

10^6: 1 mmol/L =10^6 nmol/L.

Note:

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The valid of kit is 12 months.
4. Do not use components from different batches of kit.
5. If the lactase activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S).