Disaccharidase test kit

(Cat/No.:BC119 Size:25T ~ 100T)

1. Measurement Principle (Lactase Assay)

Lactase acts on its substrate to produce a monosaccharide, which, under the action of its oxidase, produces hydrogen peroxide. The hydrogen peroxide combines with a chromogenic agent to produce a red product. The activity of lactase is determined by measuring the optical density value using a spectrophotometer.

2. Composition and preparation

Reagent 1: 1 bottle of powder, 1 bottle of 10mL diluent, colorless and transparent liquid; store at 2–8℃. When using, add 10mL of diluent to the powder in Reagent 1, dissolve thoroughly to prepare the substrate solution, and store at 2–8℃ **.**

Reagent 2: Terminator 5mL x 1 bottle, store at 2-8℃.

Reagent 3: 50mL liquid × 1 bottle, store at 2 ~ 8℃.

Glucose standard solution: 5.55 mmol/L, liquid, store at 2–8℃. **When needed, dilute with distilled water at a volume ratio of 1:2 to prepare a 1.85 mmol/L glucose standard solution**

3. Operation Table

1. Sample processing:

① **Liquid samples:** Measure directly. If the sample exceeds the linear range, dilute it with physiological saline before measurement.

② **Tissue Samples:** Accurately weigh the tissue and add 9 times its **volume of homogenizing medium at a weight (g):volume (mL) ratio of 1:9.** Homogenize mechanically under ice-water bath conditions, centrifuge at 2500 rpm for 10 minutes, and collect the supernatant for analysis. **[Note]: The homogenizing medium can be phosphate buffer (0.1 mol/L pH 7.4) or physiological saline (0.9%) .**

③ **Cell samples:**

A. Cell collection: Take out the prepared cell suspension, centrifuge at 1000 rpm for 10 minutes, discard the supernatant and keep the cell pellet; wash 1 to 2 times with isotonic buffer (recommended 0.1 mol/L, pH 7-7.4 phosphate buffer) , centrifuge at 1000 rpm for 10 minutes, discard the supernatant and keep the cell pellet;

B. Cell Disruption: Add 0.2–0.3 mL of homogenization medium **(0.1 mol/L pH 7–7.4 phosphate buffer or physiological saline is recommended)** for homogenization. Sonicate under ice-water bath conditions (power: 300W, 3–5 seconds/cycle, 30-second interval, repeated 3–5 times) or homogenize manually. The prepared homogenate can be directly analyzed without centrifugation. Alternatively, lysis buffer (Triton X-100 is recommended, 1–2%, lysis for 30–40 minutes) can be used.

The lysed liquid can be directly analyzed without centrifugation.

[Note]: A cell density of 1 million cells/mL or higher is recommended. The disrupted liquid can be observed under a microscope to check for complete cell disruption.

2. Enzymatic reaction: (can be performed using centrifuge tubes)

	Assay tube	Control tube
Sample (μL)	25	
Substrate (μL)	50	50
Mix well and incubate at 37°C for 20 minutes.		
Terminator (μL)	25	25
Sample (μL)		25
Mix well, centrifuge at 4000 rpm for 10 minutes, and collect **the supernatant** for color development.		

3. Colorimetric reaction: (First type) (ELISA reader colorimetric measurement) (100T)

	blank Tube	Standard Tube	Assay Tube	Control Tube
Distilled water (μL)	8			
1.85 mmol/L glucose standard solution (μL)		8		
Supernatant (μL)			8	8
Reagent 3	200	200	200	200
Gently shake the plate and incubate at 37°C for 15 minutes. Read the value using a microplate reader at 505 nm.				

4.Colorimetric reaction: (Second type) (Spectrophotometer colorimetry) (25T)

	Blank tube	Standard Tube	Assay Tube	control Tube
Distilled water (μL)	40			
1.85 mmol/L glucose standard solution (μL)		40		
Supernatant (μL)			40	40
Reagent 3	1000	1000	1000	1000
Mix well, incubate at 37°C for 15 minutes, and measure the color at 505 nm using a spectrophotometer with distilled water as the zero point.				

[Note]: Before conducting the formal experiment, take individual samples (one from the normal group and one from the model group) for a concentration gradient test (dilution factor can be 5 times, 10 times, 20 times or others) to determine the optimal sampling concentration (at the optimal concentration, the measured OD - control OD should be close to the standard OD; of course, if the enzyme activity of the sample itself is low, direct measurement of the original solution can even increase the sample size of the enzyme-catalyzed reaction or extend the enzyme-catalyzed reaction time).

4. Formula

1. Liquid sample calculation:

Unit definition:

Under the conditions of 37°C and pH 6.0, one enzyme activity unit (U) is defined as the amount of enzyme that hydrolyzes 1 nmol of lactose per minute in 1 mL of sample.

Calculation formula:

$$\text{Lactase Activity (U/mL)} = \frac{A_{\text{Assay}} - A_{\text{Control}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times C_{\text{Standard}} \times \frac{V_{\text{Total Reaction}}}{V_{\text{Sample}}} \div T \times 10^6$$

C_{Standard}: Concentration of standard solution, $1.85\ \text{mmol/L}$

$V_{\text{Total Reaction}}$: Total volume of enzymatic reaction system, $0.1 \times 10^{-3}\ \text{L}$

V_{Sample}: Volume of sample added, $0.025\ \text{mL}$

T: Enzymatic reaction time, $20\ \text{minutes}$

10^6: Conversion factor for $\text{mmol} \rightarrow \text{nmol}$

2. Tissue (or cell) sample calculation

Unit definition: Under the conditions of 37℃ and pH 6.0, one unit of enzyme activity is defined as the hydrolysis of 1 nmol of lactose per minute by one milligram of protein tissue.

Calculation formula:

$$\text{Lactase Activity (U/mgprot)} = \frac{A_{\text{Assay}} - A_{\text{Control}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times C_{\text{Standard}} \times \frac{V_{\text{Total Reaction}}}{T \times C_{\text{pr}} \times V_{\text{Sample}}} \times 10^6$$

C_{Standard}: Concentration of standard solution, $1.85\ \text{mmol/L}$

$V_{\text{Total Reaction}}$: Total volume of enzymatic reaction system, $0.1 \times 10^{-3}\ \text{L}$

V_{Sample}: Volume of sample added, $0.025\ \text{mL}$

T: Enzymatic reaction time, $20\ \text{minutes}$

C_{pr}: Protein concentration of sample, mgprot/mL (prot = protein)

10^6: Conversion factor for $\text{mmol} \rightarrow \text{nmol}$

V. Calculation Examples:

Freshly prepared 10% porcine jejunal mucosa homogenate was taken and processed according to

the operating table. The OD values were measured using a microplate reader. The results showed:

blank well OD 0.0695; standard well OD 0.3025; test tube OD 0.1088; control well OD 0.0715. The protein content of the 10% porcine jejunal mucosa homogenate was 2.8632 mg/mL. The calculations are as follows:

$$\text{Lactase Activity (U/mgprot)} = \frac{0.1088-0.0715}{0.3025-0.0695} \times \frac{1.85\times0.1\times10^{-3}}{20\times2.8632\times0.025} \times 10^{6} = 20.688 \text{ U/mgprot}$$