(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

β-glucuronidase (Endogenous) Assay Kit

Catalog No.: BC00218

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	β-glucuronidase (Endogenous) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue
Assay Type	Quantitative
Detection Instrument	Microplate reader (540 nm)

Product Introduction

β-Glucuronidase (β-GD) is widely present in animal tissues. As a matrix-degrading enzyme involved in tumor invasion and metastasis, it physiologically hydrolyzes steroid glucuronides and acid mucopolysaccharides. This enzyme is abundant in hepatocytes as well as gastric cancer tissues. The detection of β-GD activity in gastric juice is of great significance for gastric cancer research.

Principle

β-Glucuronidase acts on the specific substrate to release free phenolphthalein. The enzyme activity can be determined by colorimetry based on the quantity of free phenolphthalein.

Components

No.	Components	Size	Notes
Reagent 1	Powder	1 vial	Store at below -20°C.
	Diluent	5 mL	Store at 4°C.
Before use, add the entire bottle of diluent to each powder vial to prepare Working Solution 1. Mix thoroughly and prepare freshly before use.			
Reagent 2	Chromogenic reagent	40 mL	Store at 4°C.
Reagent 3	Standard powder	1 vial	Store at 4°C and protect from light.

	Standard solvent	5 mL	Store at 4°C.
Before use, dissolve one vial of standard powder with 1 mL of standard solvent to prepare 10 μmol/mL phenolphthalein standard solution and mix thoroughly. Store the prepared solution at 4°C. Then dilute the 10 μmol/mL standard solution 5-fold (1:4) with standard solvent to obtain 2 μmol/mL phenolphthalein standard solution. Prepare freshly before use.			
Consumable 1	Microplate	1 plate	Store at room temperature.
Consumable 2	Plate Sealer	2 pieces	Store at room temperature.

Storage

The kit can be stored for 3 months under specified conditions.

Preparation

Equipment and reagents required: Microplate reader (wavelength: 540 nm), 96-well plate, 37 °C water bath or incubator, tabletop centrifuge, pipettes of various specifications, double distilled water, 0.9% normal saline or 0.1M PBS, vortex mixer, test tubes or centrifuge tubes, protein assay reagents (available from our company).

- **Sample requirements**

  Tissue: Accurately weigh the tissue sample. Add 9 volumes of normal saline at a ratio of tissue weight (g) to volume (mL) = 1:9, then homogenize under low temperature (0-4 °C). Centrifuge at 4000 rpm for 10 minutes, and collect the supernatant for testing.

Operation process

Can be performed in 0.5 mL or 1.5 mL centrifuge tubes.

	Blank well	Standard well	Assay well
Double distilled water (μL)	10		
2 μmol/mL phenolphthalein standard solution (μL)		10	
Supernatant (μL)			10
Reagent 1 Working Solution (μL)	50	50	50
Mix well and incubate at 37 °C for 1 hour.			
Reagent 2 (μL)	400	400	400
Mix thoroughly, then centrifuge at 8000 rpm for 10 minutes. Transfer 200 μL of the supernatant from each tube to a 96-well plate, and measure the absorbance (A) of each well using a microplate reader at 540 nm.			

Calculation

1. Calculation for liquid samples such as serum

   Definition: One unit (U) of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μmol phenolphthalein per minute per milligram of tissue protein at 37°C.

$$\beta - \text{Glucuronidase Activity (U/mg)} = \frac{A_{Assay} - A_{Blank}}{A_{Standard} - A_{Blank}} \times C_{Standard} \div T \div Cpr$$

Annotation:

$C_{Standard}$: Standard solution concentration, 2 μmol/mL

Cpr: Tissue homogenate protein concentration, mg/mL

T: Reaction time, 60 min

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. Prepare reagents strictly in accordance with the instructions and protect from light.
4. Conduct pre-experiments for tissue homogenate in advance to determine the optimal sampling concentration (see the appendix).

Appendix

Preparation of β-GD sample curve: Dilute the freshly prepared 10% duck liver homogenate with normal saline at 2-fold, 4-fold, 8-fold, 16-fold and 32-fold dilutions, then proceed with the following experiment.

	Blank well	**Assay well**
Double distilled water (μL)	10	
Supernatants of homogenates at different concentrations (μL)		10
Reagent 1 Working Solution (μL)	50	50
Mix well and incubate at 37 °C for 1 hour.		
Reagent 2 (μL)	400	400
Mix thoroughly and centrifuge at 3500 rpm for 10 minutes. Transfer 200 μL of supernatant from each tube to a 96-well plate, then measure the absorbance (A) of each well with a microplate reader at 540 nm.		

Test Results and Data:

Homogenate concentration %	0	0.3725	0.725	1.25	2.5	5	10
Absorbance	0.0462	0.0711	0.0963	0.1387	0.2150	0.3162	0.4037
Absolute absorbance value	0	0.0249	0.0501	0.0925	0.1688	0.2700	0.3575

There is a curvilinear relationship between homogenate concentration and absorbance of the samples. The optimal sampling concentration corresponds to the concentration within the absorbance range of 0 to 0.200, where a linear proportional relationship is observed. In other words, refer to sample concentrations with an absolute absorbance below 0.2 for the optimal sampling concentration.