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CheKine™ Micro β-glucuronidase (β-GD) Activity Assay Kit

Cat #: KTB1327 Size: 48 T/48 S 96 T/96 S

	Micro β-glucuronidase (β-GD) Activity Assay Kit		
REF	**Cat #:** KTB1327	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

β-glucuronidase (β-GD), a matrix degradation enzyme involved in tumor invasion and metastasis, is widely found in animal tissues. It has physiological functions such as hydrolyzing sterol glucuronic acid and acidic mucopolysaccharide. The content of this enzyme is high in hepatocytes. In addition, it is rich in gastric cancer, so the determination of β-GD activity in gastric juice is of great significance for the study of gastric cancer. CheKine™ Micro β-glucuronidase (β-GD) Activity Assay Kit can detect animal tissues. In this kit, β-GD catalyzed phenol β-D-glucuronic acid to produce free phenolphthalein, and the enzyme activity was determined by determining the content of phenol.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60×2 mL	4℃
Reagent Ⅰ	1.2 mL	2.4 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅲ	9 mL	18 mL	4℃
Standard	1 mL	2 mL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 540 nm
- 96-well microplate or microquartz cuvette, precision pipettes, disposable pipette tips
- Water bath, cryogenic centrifuge,1.5 mL EP tube
- Deionized water
- Mortar or homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Reagent II: Prepared before use. Add 1.2 mL deionized water to 48 T and 2.4 mL deionized water to 96 T, dissolve thoroughly. Unused reagents can be stored at -20℃ for 1 month, protected from light.
Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Standard: Ready to use as supplied.1 μmol/mL standard reserve liquid. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.
Animal Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Preheat Reagent Ⅰ, Ⅱ and Ⅲ at 37℃ (mammals) or 25℃ (other species) for 10 min.
3. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	10
Standard	0	10	0
Deionized Water	10	0	0
Reagent Ⅰ	20	20	20
Reagent Ⅱ	20	20	20
Mix well, incubate for 30 min at 37℃.			
Reagent Ⅲ	150	150	150

1. Mix thoroughly, measure the absorbance value at 540 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Standard Well and Blank Well only need to be done once or twice. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.02, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculation of β-GD activity:

(1) Calculated by protein concentration

Active unit definition: The production of 1 nmol of phenolphthalein produced per milligram of protein per hour was defined as one unit of enzyme activity.

$$\beta\text{-GD(U/mg prot)}=C_{Standard}\times V_{Sample}\times \Delta A_{Test}\div \Delta A_{Standard}\div (V_{Sample}\times Cpr)\div T\times 1{,}000=\mathbf{2{,}000\times \Delta A_{Test}\div \Delta A_{Standard}\div Cpr}$$

(2) Calculated by fresh weight of samples

Active unit definition:The production of 1 nmol of phenolphthalein produced per gram tissue per hour was defined as one unit of enzyme activity.

$$\beta\text{-GD(U/g fresh weight)}=C_{Standard}\times V_{Sample}\times \Delta A_{Test}\div \Delta A_{Standard}\div (W\times V_{Sample}\div V_{Total\ sample})\div T\times 1{,}000=\mathbf{2{,}000\times \Delta A_{Test}\div \Delta A_{Standard}\div W}$$

$C_{Standard}$: the concentration of the standard, 1 μmol/mL; $V_{Sample:}$ sample volume added, 0.01 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 0.5 h; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; 1,000, conversion coefficient, 1 μmol=1,000 nmol

Precautions

The protein concentration of the sample needs to be determined by yourself. Since Extraction Buffer contains a relatively high protein concentration (about 1 mg/mL), the protein concentration of Extraction Buffer must be deducted when measuring the protein concentration of the sample.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

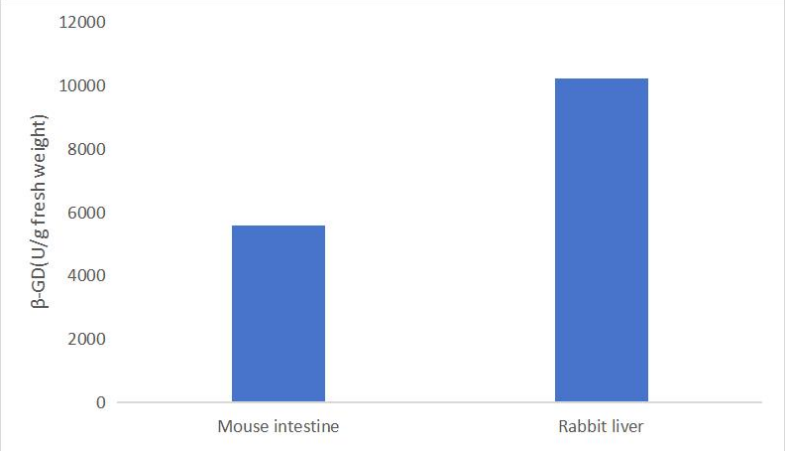

Figure 1. Determination β-GD activity in mouse intestine and rabbit liver by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB3030	CheKine™ Micro Alcohol Dehydrogenase (ADH) Activity Assay Kit
KTB1560	CheKine™ Micro Alcohol Acyltransferase (AAT) Activity Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.