Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro β-Galactosidase (β-GAL) Activity Assay Kit

Cat #: KTB1324 Size: 48 T/96 T

	Micro β-Galactosidase (β-GAL) Activity Assay Kit		
REF	**Cat #:** KTB1324	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

β-Galactosidase (β-GAL, EC 3.2.1.23) is a hydrolytic enzyme widely present in animals, plants, microorganisms, and cultured cells, capable of catalyzing β-In galactoside compounds β-Hydrolysis of galactoside bonds, in addition to the function of transgalactoside. β-GAL can not only release stored energy for rapid plant growth, but also release free galactose through normal polysaccharide metabolism, cell wall component metabolism, and hydrolysis of polysaccharides, glycoproteins, and terminal galactose residues during cell wall degradation during aging. β-GAL can decompose p-nitrobenzene-β-D-galactopyranoside generates p-nitrophenol, which has a maximum absorption peak at 400 nm, and is calculated by measuring the rate of increase in absorbance value β-GAL activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1	1	-20℃, protected from light
Reagent Ⅱ	2 mL	4 mL	4℃
Reagent Ⅲ	7.5 mL	15 mL	4℃
Standard	1 mL	1 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 400 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, ice maker, centrifuge
- Deionized water
- Mortar or homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Prepared before use. Add 1.25 mL deionized water for 48 T and 2.5 mL deionized water for 96 T to fully dissolve. Unused reagents should be packaged at -20℃ and stored in dark for 4 weeks and avoid repeated freeze-thaw cycles.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Standard: 5 μmol/mL p-nitrophenol standard solution. Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Standard preparation: Use 5 μmol/mL p-nitrophenol standard solution, prepare standard curve dilution as described in the table.

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (nmol/mL)
Std.1	40 μL 5 μmol/mL Standard	960	200
Std.2	500 μL of Std.1 (200 nmol/mL)	500	100
Std.3	500 μL of Std.2 (100 nmol/mL)	500	50
Std.4	500 μL of Std.3 (50 nmol/mL)	500	25
Std.5	500 μL of Std.4 (25 nmol/mL)	500	12.5
Std.6	500 μL of Std.5 (12.5 nmol/mL)	500	6.25
Std.7	500 μL of Std.6 (6.25 nmol/mL)	500	3.125
Blank	0	500	0 (Blank Well)

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

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.

Weigh 0.1 g tissue sample, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 15,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 400 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in the 96-well plate or microglass cuvette):

Reagent	Test Well (μL)	Control Well (μL)	Standard Well (μL)
Reagent I	25	0	0
Deionized water.	0	25	0
Reagent II	35	35	0
Sample supernatant	10	10	0
Quickly mix well and incubate at 37℃ for 30 min			
Standard	0	0	70

Reagent III	130	130	130

3. Mix thoroughly and measure the absorbance value at 400 nm. The absorbance of test well, control well, standard well and blank well were recorded as A_{Test}, $A_{Control}$, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Each test well needs to be equipped with a control well, standard curve and blank well only need to be done once or twice. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If A_{Test} is greater than 0.7, the sample supernatant can be further diluted by Extraction Buffer, and the calculation result should be multiplied by the dilution multiple.

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.

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation y=kx+b, and bring the ΔA_{Test} into the equation to get the x value (nmol/mL).

2. Calculation of β-GAL activity:

(1) Calculated by protein concentration

Active unit definition: The production of 1 nmol p-nitrophenol per min in 1 mg tissue protein is defined as a unit of enzyme activity.

β-GAL (U/mg prot)=$(x\times V_{Total})\div(V_{Sample}\times Cpr)\div T$**$=0.233\times x\div Cpr$**

(2) Calculated by sample fresh weight

Active unit definition: The production of 1 nmol p-nitrophenol per min in 1 g tissue is defined as a unit of enzyme activity.

β-GAL (U/g fresh weight)=$(x\times V_{Total})\div(W\times V_{Sample}\div V_{Total\ Sample})\div T$**$=0.233\times x\div W$**

V_{Total}: total reaction volume, 0.07 mL; V_{Sample}: sample volume added, 0.01 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; W: sample weight, g; T: reaction time, 30 min.

Typical Data

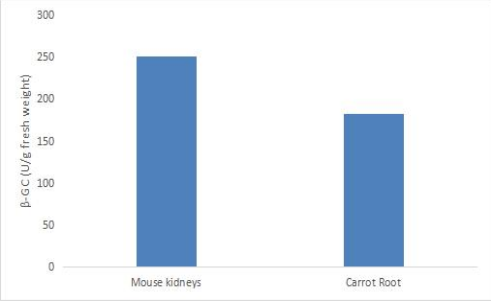

Figure 1. Determination β-GAL activity in mouse kidneys and carrot root by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.