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CheKine™ Micro α-Glucosidase Activity Assay Kit

Cat #: KTB1015 Size: 96 T/48 S

	Micro α-Glucosidase Activity Assay Kit		
REF	**Cat #:** KTB1015	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells, Serum (Plasma)		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

α-Glucosidase (3.2.1.20, EC) widely exists in animals, plants, microorganisms and cultured cells. It catalyzes the hydrolysis of α-glucosidic bonds between aryl or hydrocarbyl groups and glycosyl groups to produce glucose, which is not only related to the relaxation or reinforcement of cell walls, but also closely related to cell recognition and the production of some signal molecules. α-GC decomposes p-nitrobenzene-α-D-glucopyranoside to generate p-nitrophenol, which has the maximum absorption peak at 400 nm. α-GC activity is calculated by measuring the rising rate of absorbance value.

Materials Supplied and Storage Conditions

Kit components	Size (96 T)	Storage conditions
Extraction Buffer	60×2 mL	4℃
Reagent Ⅰ	Powder×1 vial	-20℃, protected from light
Reagent Ⅱ	24 mL	4℃
Reagent Ⅲ	22 mL	4℃
Standard	1 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 visible spectrophotometer capable of measuring absorbance at 400 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic incubator,thermostat water bath, ice maker centrifuge
- Deionized water
- 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 12 mL deionized water to fully dissolve. Unused reagents should be aliquoted and stored protected from light at 20℃ for up to one month. 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℃.

Note: Standard has a pungent odor, so it is recommended to experiment in a fume hood.

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	20 μL 5 μmol/mL Standard	980	100
Std.2	500 μL of Std.1 (100 nmol/mL)	500	50
Std.3	500 μL of Std.2 (50 nmol/mL)	500	25
Std.4	500 μL of Std.3 (25 nmol/mL)	500	12.5
Std.5	500 μL of Std.4 (12.5 nmol/mL)	500	6.25
Std.6	500 μL of Std.5 (6.25 nmol/mL)	500	3.125
Std.7	500 μL of Std.6 (3.125 nmol/mL)	500	1.563
Blank	0	500	0（Blank Tube）

Notes: Always prepare fresh standards per use; Diluted Standard 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.

1. Tissues: Weigh 0.1 g tissue, 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.

2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 15,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3. Serum (Plasma): Test directly. If the solution is turbid, the supernatant should be taken for determination after centrifugation.

Note: 1. 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.

2. The animal and plant tissues samples extracted by this kit can also be used for the determination of KTB1322, KTB1323 and KTB1324.

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 1.5 mL EP tube):

Reagent	Test Tube (μL)	Control Tube (μL)	Standard Tube(μL)
Reagent I	120	0	0

Reagent II	150	150	0
Deionized water	0	120	0
Sample supernatant	30	30	0
Fully mix well, put it in an accurate water bath at 37℃ for 30 min, then immediately put it in a water bath at 95℃ for 5 min (cover tightly to prevent water loss), fully mix well after cooling (to ensure constant concentration), centrifuge at 8,000 g at 4℃ for 5 min, and take the supernatant (add the following reagents to a 1 mL microglass cuvette or a 96-well plate)			
Standard	0	0	70
supernatant	70	70	0
Reagent III	130	130	130

3. After fully mixing and standing at room temperature for 2 min, the absorbance was measured at 400 nm. The absorbance of test well, control well, standard well were recorded as A_{Test}, $A_{Control}$ and $A_{Standard}$. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Standard curve and blank tube 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 0.005, the sample volume can be appropriately increased, and the calculation formula should be adjusted accordingly. If ΔA_{Test} is greater than 0.4, 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, and bring the ΔA_{Test} into the equation to get the x value (nmol/mL).

2. Calculation of α-GC 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.

α-GC (U/mg prot)=$(x\times V_{Total})\div(V_{Sample}\times Cpr)\div T$**=x÷Cpr÷3**

(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.

α-GC (U/g fresh weight)=$(x\times V_{Total})\div(W\times V_{Sample}\div V_{Total\ Sample})\div T$**=x÷W÷3**

(3) Calculated by cell number

Active unit definition: The production of 1 nmol p-nitrophenol per min in 10^4 cells is defined as a unit of enzyme activity.

α-GC (U/10^4 cell)=$(x\times V_{Total})\div(500\times V_{Sample}\div V_{Total\ Sample})\div T$**=x÷500÷3**

(4) Calculated by volume of liquid samples

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

α-GC (U/mL)=$(x\times V_{Total})\div(V_{Sample}\div V_{Total\ Sample})\div T$**=x÷3**

V_{Total}: total reaction volume, 0.3 mL; V_{Sample}: sample volume added, 0.03 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; W: sample weight, g; 500: Total number of cells, 5×10^6. T: reaction time, 30 min.

Typical Data

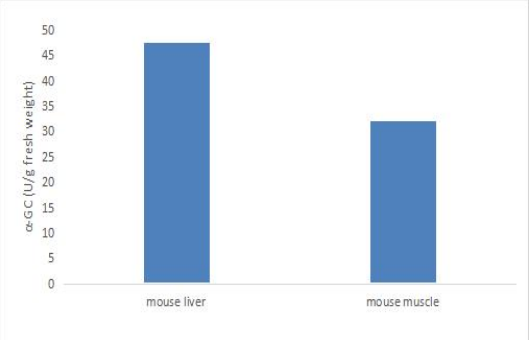

Figure 1. Determination α-GC activity in mouse liver and muscle by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1100	CheKine™ Micro Lactic Acid（LA）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. For your safety and health, please wear a lab coat and disposable gloves.