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CheKine™ Micro Glycosylase Activity Assay Kit

Cat #: KTB1570 Size: 48 T/96 T

	Micro Glycosylase Activity Assay Kit		
REF	**Cat #:** KTB1570	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Bacteria or Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Glycosylase(EC3.2.1.3) also known as γ-amylase, which is an extracellular enzyme that is secreted by a series of microorganisms and has exonuclease activity. The main role of Glycosylase is to hydrolyze α-1,4 glycosidic bonds in sequence from non-reducing ends on the carbon chains such as starch, dextrin, glycogen, etc., and cut off each glucose unit. For amylopectin, when it encounters a branch point, it can also hydrolyze α-1,6 glycosidic bonds, thereby hydrolyzing all amylopectin to glucose. It is one of the important industrial enzyme preparations in China, and is widely used in alcohol, liquor, antibiotics, amino acids, organic acids, glycerol, starch sugar and other industries. CheKine™ Micro Glycosylase Activity Assay Kit can be used to detect biological samples such as animal and plant tissues, bacteria or cells, serum or plasma. In the kit, glycosylase converts soluble starch to glucose. Under the alkaline condition, 3.5-Dinitrosalicylic acid is reduced to brown-red amino compound by co-heating with glucose. The brown-red amino compound has a maximum absorption peak at 540 nm and the absorbance ratio is in direct proportion to the contents of glucose. In this kit, the glycosylase activity is quantified by measuring the color development at 540 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	5 mL	10 mL	4℃
Reagent Ⅱ	5 mL	10 mL	4℃, protected from light
Standard	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath pot, cryogenic centrifuge

- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

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

Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃. (If precipitation occurs, it can be heated at 90℃ for 5 min before dissolution)

Reagent II: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Stardard： Prepared before use. Add 1 mL Extraction Buffer to fully dissolve to generate a 10 mg/mL glucose standard solution, store at 4℃ and use within 2 weeks.

Standard preparation: Use the 10 mg/mL glucose standard solution and further dilute it to the standard as shown in the following table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (mg/mL)
Std.1	200 μL 10 mg/mL Standard	800	2.0
Std.2	150 μL of Std.1 (2.0 mg/mL)	50	1.5
Std.3	300 μL of Std.1 (2.0 mg/mL)	300	1.0
Std.4	320 μL of Std.3 (1.0 mg/mL)	80	0.8
Std.5	200 μL of Std.4 (0.8 mg/mL)	200	0.4
Std.6	200 μL of Std.5 (0.4 mg/mL)	200	0.2
Std.7	200 μL of Std.6 (0.2 mg/mL)	200	0.1

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.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Bacteria or Cells: Collect 5×10^6 bacteria or cells into the centrifuge tube, wash bacteria or cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 3 min (power 30% or 300 W, ultrasonic 3 s, interval 7 s). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum or other Liquid samples: Direct detection.

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. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)

Sample	0	0	10	0
Inactivated sample	0	0	0	10
Standard	0	10	0	0
Deionized water	10	0	0	0
Reagent Ⅰ	100	100	100	100
Mix thoroughly, put in 40℃ water bath for 20 min				
Reagent Ⅱ	90	90	90	90

3. Mix thoroughly, boiling water bath for 5 min (cover tightly to prevent water loss), add 200 μL to micro glass cuvette/96 well flat-bottom plate, detect the absorbance at 540 nm after cooling with running water. The Blank Well is recorded as A_{Blank}, the standard Well is marked as $A_{Standard}$, the Test Well is marked as $A_{Test,}$ and the Control Well is marked as $A_{Control.}$ Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank Well and the Standard Well only need to be done 1-2 times. 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 0.4, increase the sample quantity appropriately. If ΔA_{Test} is greater than 2, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. Samples with high protein content are recommended to be diluted with Extraction Buffer before treatment and detection.

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 and obtain the standard equation y=kx+b. The determination of ΔA_{Test} is brought into the equation to get x(mg/mL).

2. Calculation of the Glycosylase activity

(1) Calculated by sample protein concentration

Unit definition: At 40℃ and pH4.6, the amount of enzyme required to decompose soluble starch to produce 1 mg glucose per milligram per min is one unit of enzyme activity.

Glycosylase (U/mg prot)=$x\times V_{Reaction\ volume}\div(V_{Sample}\times Cpr)\div T$**=5.5x÷Cpr**

(2) Calculated by fresh weight of samples

Unit definition: At 40℃ and pH4.6, the amount of enzyme required to decompose soluble starch to produce 1 mg glucose per gram of tissue per min is one unit of enzyme activity.

Glycosylase (U/g fresh weight)=$x\times V_{Reaction\ volume}\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**=5.5x÷w**

(3) Calculated by bacteria or cells

Unit definition: At 40℃, pH4.6 conditions, the amount of enzyme required for each 10^4 bacteria or cells to decompose soluble starch to produce 1 mg of glucose per min is one unit of enzyme activity.

Glycosylase ($U/10^4$)=$x\times V_{Reaction\ volume}\div(V_{Sample}\times n\div V_{Total\ sample})\div T$**=0.55x÷n**

(4) Calculated by volume of liquid samples

Unit definition: At 40℃, pH4.6 conditions, per milliliter of liquid per min to decompose soluble starch to produce 1 mg of glucose required enzyme activity unit.

Glycosylase (U/mL)=$x\times V_{Reaction\ volume}\div V_{Sample}\div T$**=0.55x**

$V_{Reaction\ volume}$: Total volume of reaction, 0.11 mL; V_{Sample}: Added sample volume to the reaction system, 0.01 mL; $V_{Total\ sample}$: Added the Extraction Buffer volume, 1 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; T: Reaction time, 20 min; n: Number of bacteria or cells, ten thousand.

Typical Data

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

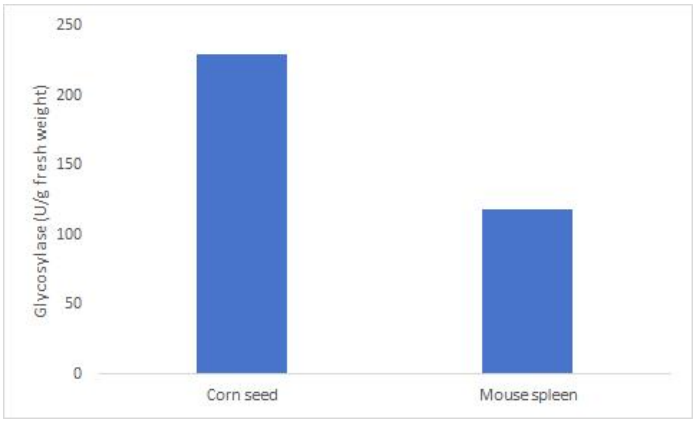

Figure 1. Determination of glycosylase activity in corn seed and mouse spleen by this kit.

Recommended Products

Catalog No.	Product Name
KTB1370	CheKine™ Micro α-Amylase Activity Assay Kit
KTB1380	CheKine™ Micro β-Amylase Activity Assay Kit
KTB1390	CheKine™ Micro Starch Branching Enzyme(SBE) Activity Assay Kit

Disclaimer

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