CheKine™ Micro Soil β-Glucosidase (S-β-GC) Activity Assay Kit

Cat #: KTB4022 Size: 48 T/24 S 96 T/48 S

	Micro Soil β-Glucosidase (S-β-GC) Activity Assay Kit		
REF	**Cat #:** KTB4022	LOT	**Lot #:** Refer to product label
	Applicable sample: Soil sample		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Soil β-glucosidase (S-β-GC) is capable of catalyzing the hydrolysis of glycosidic bonds between aryl or alkyl groups and sugar moieties, producing glucose. It is an important component of the cellulose-degrading enzyme system and plays a significant physiological role in carbohydrate metabolism by soil microorganisms.CheKine™ Micro Soil β-Glucosidase (S-β-GC) Activity Assay Kit provides a simple, convenient, and rapid method for detecting S-β-GC activity suitable for soil samples. The principle behind this assay is that S-β-GC can catalyze the conversion of p-nitrophenyl-β-D-glucopyranoside to p-nitrophenol, which exhibits characteristic optical absorption at 400 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅱ	14 mL	28 mL	4℃
Reagent Ⅲ	13 mL	26 mL	4℃
Standard	1 mL	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
- Oven, 30-50 mesh sieve, centrifuge, constant temperature water bath, analytical balance
- Deionized water, toluene

Reagent Preparation

Reagent Ⅰ: Prepared before use. Add 5 mL of deionized water to the 48 T, and add 10 mL of deionized water to the 96 T 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 mM 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 mM p-nitrophenol standard solution, prepare standard curve dilution as described in the table.

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (μM)
Std.1	20 μL 5 mM Standard	980	100
Std.2	500 μL of Std.1 (100 μM)	500	50
Std.3	500 μL of Std.2 (50 μM)	500	25
Std.4	500 μL of Std.3 (25 μM)	500	12.5
Std.5	500 μL of Std.4 (12.5 μM)	500	6.25
Std.6	500 μL of Std.5 (6.25 μM)	500	3.125
Std.7	500 μL of Std.6 (3.125 μM)	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: Fresh samples are recommended.

Fresh soil samples should be air-dried naturally or dried in an oven at 37℃, then passed through a 30-50 mesh sieve.

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)
Air-dried soil sample (g)	0.02	0.02	0
Toluene	10	10	0
	Mix by shaking at RT for 15 min	Mix by shaking at 90℃ for 15 min	
Reagent Ⅰ	130	0	0
Deionized water	0	130	0
Reagent Ⅱ	160	160	0
Mix thoroughly. Incubate with shaking at 37℃ for 1 h. Then, place in a water bath at 90℃ for 5 min, ensuring the container is tightly sealed to prevent water loss. Cool under running water. Centrifuge at 10,000 g for 10 min at 25℃, and collect the supernatant. (Add the following reagents to a 1.5 mL Eppendorf tube)			
Supernatant	84	84	0

Standard	0	0	84
Reagent III	156	156	156

Mix thoroughly. After centrifuging at 10,000 g for 5 min at 25℃, transfer 200 μL of the supernatant to a 96-well plate or a micro glass cuvette. Measure the absorbance at 400 nm. Record the absorbance values as A_{Test}, $A_{Control}$, $A_{Standard}$, and A_{Blank}, respectively. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note:The standard curve needs to be determined only once, and a control well should be set up for each measurement well. 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 0.3, the sample supernatant can be further diluted by deionized water, 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 (μM).

2. Calculation of S-β-GC activity:

Active unit definition: One unit of enzyme activity is defined as the production of 1 μmol of p-nitrophenol per g of soil sample per day.

S-β-GC (U/g soil sample)=$x\times V_{Total\ reaction}\div W\div T$**$=0.0072\times x\div W$**

Where: T: Reaction time, 1 h=1/24 d; $V_{Total\ reaction}$: Total volume of reaction system, 3×10^{-4} L; W: Sample mass, 0.02 g.

Typical Data

Typical standard curve:

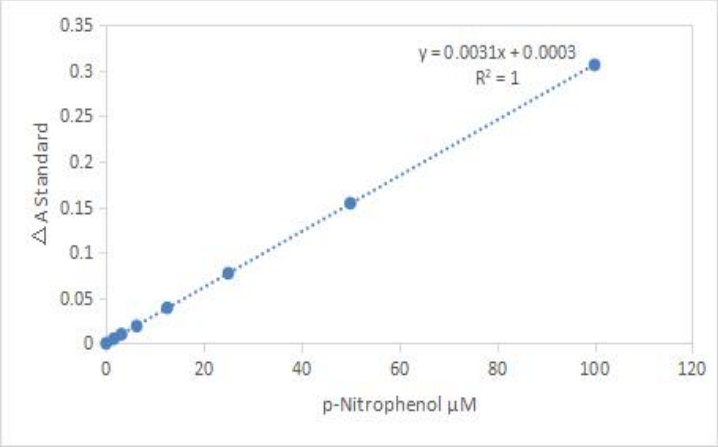

Figure1. p-Nitrophenol standard curve

Examples:

Take 0.02 g of fresh soil sample that has been dried in a 37℃ oven and use 96-well plate to calculate ΔA_{Test}=0.283-0.105=0.178, x=59.278. The content calculated according to the soil sample mass is as follows:

S-β-GC (U/g soil sample)=0.0072×59.278÷0.02=21.34 U/g

Recommended Products

Catalog No.	Product Name
KTB4023	CheKine™ Mirco Soil Peroxidase (S-POD) Activity Assay Kit
KTB4024	CheKine™ Mirco Soil Acid Protease (S-ACPT) Activity Assay Kit
KTB4025	CheKine™ Mirco Soil β-Xylosidase (S-β-XYS) 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.