



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

Cat #: KTB4019

Size: 48 T/24 S 96 T/48 S

| | | | |
|---|--|------------|--------------------------------------|
|  | Micro Soil α-Glucosidase (S-α-GC) Activity Assay Kit | | |
| REF | Cat #: KTB4019 | LOT | Lot #: Refer to product label |
| | Applicable sample: Soil sample | | |
|  | Storage: Stored at -20°C for 6 months, protected from light | | |

Assay Principle

Soil α-glucosidase (S-α-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 α-Glucosidase (S-α-GC) Activity Assay Kit provides a simple, convenient, and rapid method for detecting S-α-GC activity suitable for soil samples. The principle behind this assay is that S-α-GC can catalyze the conversion of p-nitrophenyl-α-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°C, protected from light |
| Reagent II | 14 mL | 28 mL | 4°C |
| Reagent III | 13 mL | 26 mL | 4°C |
| Standard | 1 mL | 1 mL | 4°C, 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°C 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°C.

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

Standard: 5 mM p-nitrophenol standard solution. Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

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°C, 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°C 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°C for 1 h. Then, place in a water bath at 90°C 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°C, 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°C, 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 $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Control}}$, $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$.

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 $\Delta A_{\text{Standard}}$ 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-α-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.

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

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

Typical Data

Typical standard curve:

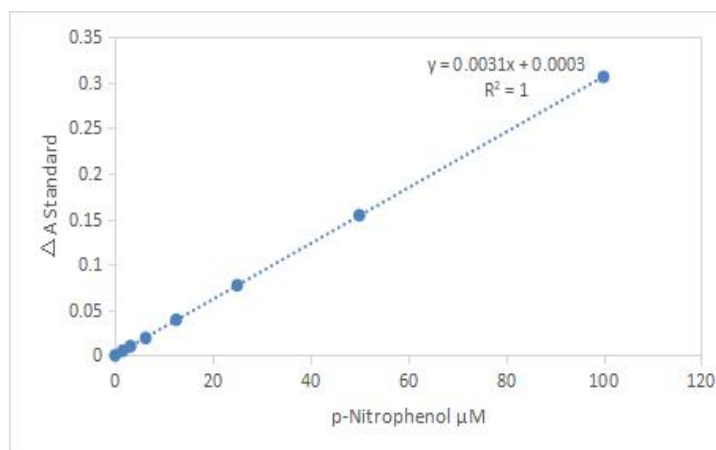


Figure1. p-Nitrophenol standard curve

Examples:

Take 0.02 g of fresh soil sample that has been dried in a 37°C oven and use 96-well plate to calculate $\Delta A_{\text{Test}} = 0.162 - 0.097 = 0.065$, $x = 20.871$. The content calculated according to the soil sample mass is as follows:

$$\text{S-}\alpha\text{-GC (U/g soil sample)} = 0.0072 \times 20.871 \div 0.02 = 7.514 \text{ U/g}$$

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.