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CheKine™ Micro Soil Neutral Invertase (S-NI) Activity Assay Kit

Cat #: KTB4062 Size: 48 T/24 S 96 T/48 S

	Micro Soil Neutral Invertase (S-NI) Activity Assay Kit		
REF	**Cat #:** KTB4062	LOT	**Lot #:** Refer to product label
	Detection range: 0.03215-1 mg/mL		**Sensitivity:** 0.03215 mg/mL
	Applicable sample: Soil sample		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Sucrose invertase (Invertase, Ivr) catalyzes the irreversible hydrolysis of sucrose into fructose and glucose, and is one of the key enzymes involved in sucrose metabolism in higher plants. Based on optimal pH, Ivr can be classified into two types: acid invertase (AI) and neutral invertase (NI). CheKine™ Micro Soil Neutral Invertase S-NI) Activity Assay Kit provides a simple, convenient, and rapid method for determining neutral invertase activity in soil samples. The principle of the assay is based on the ability of S-NI to catalyze the hydrolysis of sucrose, producing reducing sugars that subsequently react with 3,5-dinitrosalicylic acid (DNS) to form a red-brown amino compound. This compound exhibits a characteristic absorbance at 540 nm, and the absorbance value is proportional to the amount of reducing sugar produced within a certain range. The activity of S-NI is calculated based on the rate of increase in absorbance at 540 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	30 mL	60 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃
Reagent Ⅲ	10 mL	20 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃

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 ultraviolet spectrophotometer capable of measuring absorbance at 540 nm
- 96-well microplate or microquartz cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge, ice maker
- Deionized water, toluene

Reagent Preparation

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

Working Reagent Ⅱ: Prepared before use. Add 14 mL Reagent Ⅰ for 48 T and 28 mL Reagent Ⅰ for 96 T to fully dissolve. The remaining reagent can also be stored at 4℃ for 2 weeks.

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

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

Standard: Prepared before use. Add 1 mL deionized water and fully dissolve to 10 mg/mL. The remaining reagent can also be stored at 4℃ for 1 month. Use the 10 mg/mL 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	100 μL 10 mg/mL Standard	900	1
Std.2	500 μL of Std.1 (1 mg/mL Standard)	500	0.5
Std.3	500 μL of Std.2 (0.5 mg/mL Standard)	500	0.25
Std.4	500 μL of Std.3 (0.25 mg/mL Standard)	500	0.125
Std.5	500 μL of Std.4 (0.125 mg/mL Standard)	500	0.0625
Std.6	500 μL of Std.5 (0.0625 mg/mL Standard)	500	0.03125

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.

Fresh soil samples were air-dried naturally or dried in an oven at 37 ℃, and then sieved 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 540 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Blank Tube	Standard Tube	Control Tube	Test Tube
Air-dried soil sample (g)	0	0	0.05	0.05
Standard (μL)	0	400	0	0
Deionized water (μL)	400	0	0	0
Reagent Ⅰ (μL)	0	0	400	0
Working Reagent Ⅱ (μL)	0	0	0	400
Mix thoroughly, incubate at 37℃ in a water bath for 1 h, then transfer to a 95℃ water bath for 10 min (keep the tube tightly capped to prevent water loss). Afterward, cool the sample under running water and vortex thoroughly to ensure uniformity (to maintain consistent concentration). Centrifuge at 10,000 g for 10 min at 25℃, and collect the supernatant.				
Supernatant	200	200	200	200
Reagent Ⅲ (μL)	125	125	125	125

3. Mix well, bathe in water at 95℃ for 10 min (cover tightly to prevent water loss), cool down with running water and mix well, take 200 μL into 96-well microplate or microquartz cuvette, and record the absorbance value at 540 nm. The Blank Tube is recorded as A_{Blank}, the Standard Tube is marked as $A_{Standard}$, the Control Tube is marked as $A_{Control}$, and the Test Tube is marked as A_{Test}. 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, each sample assay should include a corresponding control. 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 0.004, the sample volume can be appropriately increased. If ΔA_{Test} is greater than 3.4, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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. The determination of ΔA_{Test} is substituted into the equation to get x (mg/mL).

2. Calculation of the S-NI activity

Active unit definition: The production of 1 mg reducing sugar per milligram of protein per day at 37℃ was defined as one unit of enzyme activity.

S-NI (U/g soil)=x×V÷W÷T**=9.6×x÷W**

V: Standard volume added, 0.4 mL; W: weight of soil, g; T: reaction time, 1/24 d

Precautions

If Reagent III is added and turbidity appears after 10 min of water bath at 95℃, it is recommended to centrifuge at 4℃ for 5 min at 12,000 g and then take supernatant to measure absorbance.

Typical Data

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

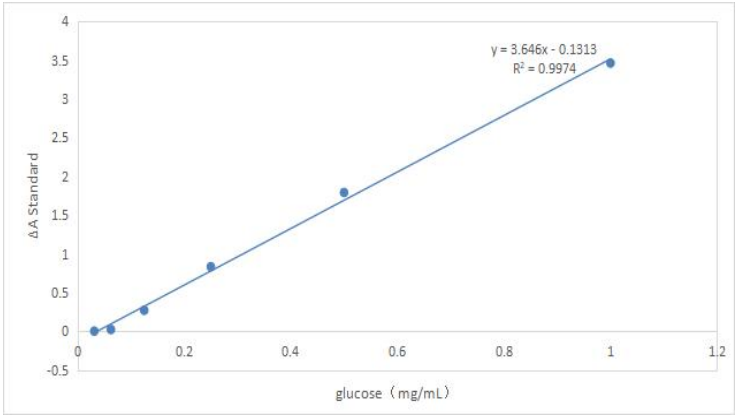

Figure 1. Glucose standard curve

Recommended Products

Catalog No.	Product Name
KTB4043	CheKine™ Micro Soil Neutral Phosphatase(S-NP) 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.