CheKine™ Mirco Soil Acid Invertase (S-AI) Activity Assay Kit

Cat #: KTB4027 Size: 48 T/48 S 96 T/96 S

	Mirco Soil Acid Invertase (S-AI) Activity Assay Kit		
REF	**Cat #:** KTB4027	LOT	**Lot #:** Refer to product label
	Detection range: 0.06-0.4 mg/mL (23.04-153.6 U/g Soil)		**Sensitivity:** 0.06 mg/mL (23.04 U/g Soil)
	Applicable sample: Soil		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

S-AI catalyzes the irreversible decomposition of sucrose into fructose and glucose at pH 4.5-5.0 (acidic), and is one of the key enzymes in soil microbial sucrose metabolism. CheKine™ Mirco Soil Acid Invertase (S-AI) Activity Assay Kit can detect soil samples. In this kit, S-AI catalyzes sucrose degradation to produce reducing sugar, which further reacts with 3, 5-dinitrosalicylate to produce brown-red amino compounds with characteristic light absorption at 510 nm, and the rate of increase of 510 nm light absorption is proportional to S-AI activity within a certain range.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	70 mL	70 mL×2	4℃
Reagent Ⅱ	Powder×1 vial	Powder×2 vials	4℃
Reagent Ⅲ	13 mL	26 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 510 nm
- 96-well microplate or microquartz cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath/metal bath, cryogenic centrifuge, thermostatic incubator
- Deionized water, 30-50 mesh sieve

Reagent Preparation

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

Reagent Ⅱ: Prepared before use. Add 25 mL Reagent Ⅰ for each bottle 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.

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)	Reagent Ⅰ Volume (μL)	Concentration (mg/mL)
Std.1	40 μL of 10 mg/mL Standard	960	0.4
Std.2	30 μL of 10 mg/mL Standard	970	0.3
Std.3	20 μL of 10 mg/mL Standard	980	0.2
Std.4	10 μL of 10 mg/mL Standard	990	0.1
Std.5	8 μL of 10 mg/mL Standard	992	0.08
Std.6	6 μL of 10 mg/mL Standard	994	0.06

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: It is recommended to use fresh soil samples.

Fresh soil samples naturally air dried or air dried in an oven at 37℃ and sieved through 30-50 mesh sieve.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 510 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 Well	Control Well	Standard Well	Blank Well
Soil Sample	0.05	0.05	0	0
Standard (μL)	0	0	400	0
Reagent Ⅰ (μL)	0	400	0	400
Reagent Ⅱ (μL)	400	0	0	0
Mix well, hold at 37℃ for 30 min, place in 100℃ boiling water bath for 10 min (cover tightly to prevent water loss), and cool down with running water.				
Supernatant (μL)	200	200	200	200
Reagent Ⅲ (μL)	100	100	100	100

3. Mix well, bathe in water at 100℃ 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 microglass cuvette, and record the absorbance value at 510 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Control Well is marked as $A_{Control}$, and the Test Well 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. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If after add Reagent Ⅲ, turbidity appears after boiling water bath for 10 min, it is recommended to 10,000 g, centrifuge at 25℃ for 5 min, and take supernatant to determine absorbance. If the ΔA_{Test} determination is greater than 2, the sample can be further diluted by Reagent Ⅰ, the calculated result is multiplied by the dilution factor, or the sample size for extraction is reduced. If the ΔA_{Test} determination is less than 0.03, increase the sample size.

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 y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is substituted into the equation to get y (mg/mL).

2. Calculation of the S-AI activity

Definition of unit: 1 mg of reducing sugar produced per g of soil per day at 37℃ is defined as a unit of enzyme activity.

S-AI (U/g soil) = $y \times V_{reaction} \div W \div T$**$=19.2 \times y \div W$**

$V_{reaction}$: The volume of the reaction system: 0.4 mL; T: Reaction time, 1/48 d; W: weight of sample, g.

Typical Data

The following data are for reference only.

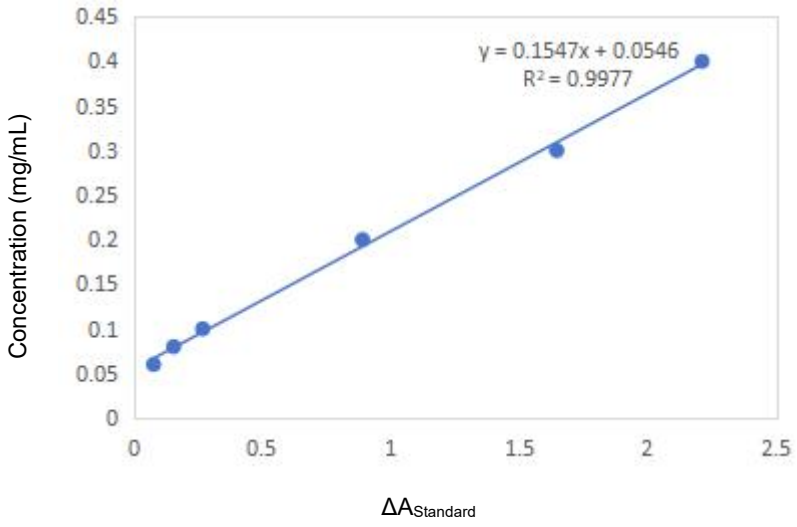

Figure 1. S-AI standard curve.

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.