CheKine™ Micro Soil Free Amino Acid (S-FAA) Content Assay Kit

Cat #: KTB4063 Size: 48 T/48 S 96 T/96 S

	Micro Soil Free Amino Acid (S-FAA) Content Assay Kit		
REF	**Cat #:** KTB4063	LOT	**Lot #:** Refer to product label
	Detection range: 1.25-20 μmol/mL		**Sensitivity:** 1.25 μmol/mL
	Applicable samples: Soil		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

As an important class of compounds in soil, amino acids account for 15%-60% of total nitrogen in soil. They are important "stores" and "sources" in the process of soil nitrogen circulation and plant nutrient supply. They can meet the nitrogen nutrition needs of crops through mineralization, and can also be directly absorbed and utilized by plants. In addition, amino acids are also important nitrogen sources for soil microorganisms and have a direct impact on the structure, number and activity of soil microbial communities. Therefore, studying the changes in amino acid content in soil is of great significance to studying soil nitrogen circulation and its impact on plant physiological and ecological changes. CheKine™ Micro Soil Free Amino Acid (S-FAA) Content Assay Kit provides a simple, convenient, and rapid method for amino acid, which is suitable for the detection of soil. The principle is that the α-amino of amino acid can react with Ninhydrin hydrate to produce blue-purple substances, which has a maximum absorption peak at 570 nm. The amino acid content of the sample can be calculated by measuring the absorbance at 570 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	3.5 mL	7 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Standard (5 mg)	Powder×1 vial	Powder×1 vial	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 570 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Centrifuge, water bath, Oscillator, 30-50 mesh sieve

• Deionized water, ethanol

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℃.

Working Reagent Ⅱ: Prepared before use. Add 1.5 mL 95% ethanol to dissolve to each Reagent Ⅱ. Unused dissolved Reagent Ⅱ can be stored at 4℃ for one week, protected from light.

Note: Reagent Ⅰ and Reagent Ⅱ is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

Standard: Prepared before use. Add 1.033 mL deionized water to dissolve. The concentration is 40 μmoL/mL. Unused dissolved Standard can be stored at 4℃ for one week, protected from light.

Standard Curve Setting: Dilute 40 μmol/mL Standard with deionized water to 20, 10, 5, 2.5, 1.25 μmoL/mL standard solution as shown in the table below.

Num.	Volume of Standard	Volume of Deionized Water (μL)	The Concentration of Standard
Std.1	100 μL of 40 μmol/mL	100	20 μmol/mL
Std.2	100 μL of Std.1（20 μmol/mL）	100	10 μmol/mL
Std.3	100 μL of Std.2（10 μmol/mL）	100	5 μmol/mL
Std.4	100 μL of Std.3（5 μmol/mL）	100	2.5 μmol/mL
Std.5	100 μL of Std.4（2.5 μmol/mL）	100	1.25 μmol/mL
Blank	0	100	0

Notes: Always prepare fresh standards per use; Diluted Standard 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. Weigh 0.2 g dried soil, add 1 mL Extraction Buffer and homogenize, oscillate for 2 h at room temperature. Centrifuge at 10,000 g for 10 min at room temperature. Use supernatant for assay.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 570 nm, visible spectrophotometer was returned to zero with deionized water.

2. Add the following reagents respectively into each EP tubes:

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Deionized Water	20	0	0
Std. with Different Concentration	0	20	0
Sample	0	0	20
Working Reagent Ⅱ	20	20	20
Reagent Ⅰ	50	50	50

3. Mix well, then cover tightly and place in boiling water for 5 min. After cooling with running water for 10 s, add 120 μL 60% ethanol. Then reverse the EP tube several times and transfer 150 μL of each reaction to a 96-well plate or microglass cuvette. Then reading the values at 570 nm. The absorbance of Blank tube, Standard tube, Test tube recorded as A_{Blank}, $A_{Standard}$ and A_{Test}

respectively. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$ (Blank tube only needs to make one tube). Be sure to finish the measurement within 30 min after color development.

Note: 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 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than $\Delta A_{Standard}$ of 20 μmol/mL, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. The reaction of proline and hydroxyproline with Ninhydrin has no absorption peak at 570 nm, therefore, the determination results at 570 nm do not contain these two amino acids.

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 (μmol/mL).

2. Calculate the content of S-FAA in soil

S-FAA (μmol/g soil)=$(x\times V_{Sample})\div(W\times V_{Sample}\div V_{Extraction\ Buffer})$**$=x\div W$**

Where: V_{Sample}: the volume of add sample volume, 0.02 mL; $V_{Extraction\ Buffer}$: the volume of add Extraction Buffer, 1 mL; W: the weight of sample, g.

Typical Data

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

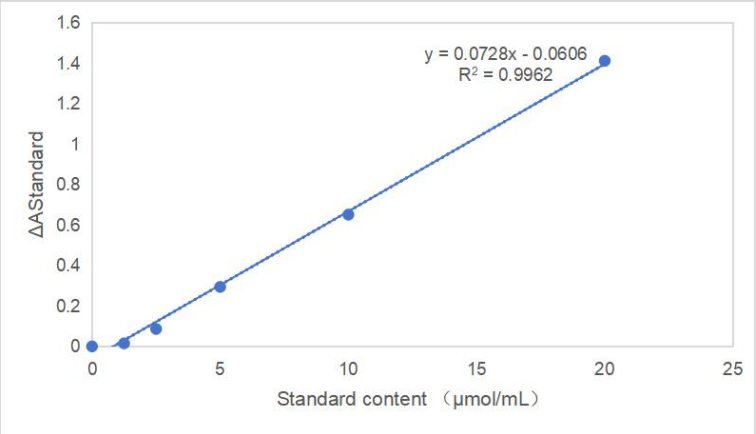

Figure 1. Standard curve of S-FAA.

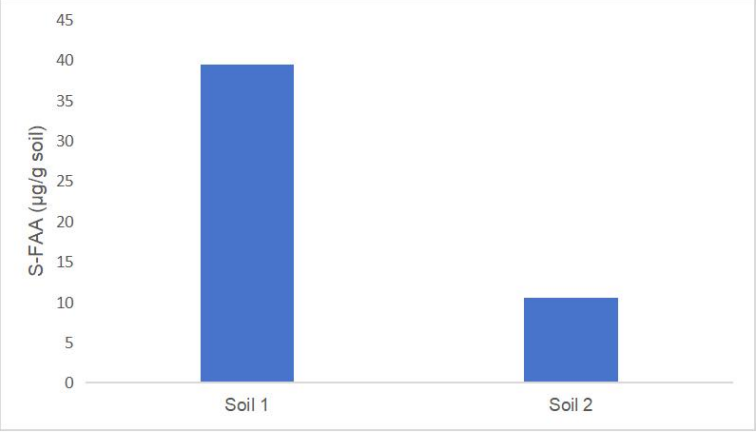

Figure 2. Determination of S-FAA in soil by this kit.

Recommended Products

Catalog No.	Product Name
KTB4012	CheKine™ Micro Soil Nitrate Nitrogen Assay Kit
KTB4014	CheKine™ Micro Acid Soil Available Phosphorous Assay Kit
KTB4041	CheKine™ Micro Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
KTB4050	CheKine™ Micro Soil Catalase (S-CAT) 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.