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

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer/Microplate reader

Cat No: BC5095

Size: 100T/96S

Components:

Reagent I: Liquid 110 mL×1, store at 4℃.

Reagent II: Liquid 12 mL×1, store at 4℃.

Reagent III: Liquid 15 mL×1, store at 4℃.

Reagent IV: Powder×2, store at 4℃. Each powder should be dissolved completely in 1 mL of distilled water before use. The unused reagent could be stored at -20℃ for 2 weeks.

Standard: Powder×1, store at 4℃. Dissolve in 4.13 mL of distilled water and prepare as 20 μmol/mL standard solution before use. The unused solution could be stored at 4℃ for 4 weeks.

Description:

As an important class of compounds, amino acids account for 15%-60% of total nitrogen in soil, which are 'sink' and 'source' in soil nitrogen cycle and plant nutrient supply. Amino acids could be absorbed directly or by mineralization to satisfy the need of plant nitrogen nutrition. Amino acids are also important nitrogen sources for soil microbes and have direct effects on soil microbial community structure, quantity and activity. Study on variation of amino acid contents in soil is of great significance to soil nitrogen cycle and plant ecophysiology.

α-Amino of amino acid could react with hydrated ninhydrin to produce blue purple compound, which has absorption peak at 570 nm, and content of amino acid is calculated by measuring absorbance of 570 nm.

Required but not provided:

Spectrophotometer/microplate reader, refrigerated centrifuge, transferpettor, water bath/dry bath, micro glass cuvette/96 well flat-bottom plate, mortar, 30-50 mesh sieve, ice and distilled water.

Protocol:

I. Sample preparation

After drying naturally or at 37℃, soil samples should be passed through 30-50 mesh sieve. Add 1 mL of Reagent I to 0.15 g of soil samples, mix thoroughly. Oscillate for 2 hour and centrifuge at 10000 rpm for 10 minutes to remove insoluble materials, and take the supernatant on ice for testing.

II. Determination procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 570 nm, set zero with distilled water.

2. Standard: Dilute 60μL of 20 μmol/mL standard solution with 140μL of distilled water to generate a 6

μmol/mL standard.

3. Add reagents as the following table.

Reagent (μL)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	40	-	-
Standard	-	40	-
Distilled water	-	-	40
Reagent II	100	100	100
Reagent III	100	100	100
Reagent IV	10	10	10

Mix thoroughly, incubate at boiling water for 15 minutes, repeatedly overthrow centrifuge several times. Centrifuge at 8000 rpm for 5 minutes, then detect the absorbance of supernatant at 570 nm. Record as A_T, A_S, A_B, $\Delta A_T=A_T-A_B$, $\Delta A_S=A_S-A_B$. Detect within 30 minutes after coloration. Standard tube and blank tube need to test once or twice.

III. Calculation

AA (μmol/g weight) $=(C_S\times V_S\times\Delta A_T\div\Delta A_S)\div(V_{SV}\div V_{ST}\times W)=6\times\Delta A_T\div\Delta A_S\div W$

C_S: Standard concentration, 6 μmol/mL;

V_S: Standard volume, 0.04 mL;

W: Sample weight, g;

V_{SV}: Sample volume, 0.04 mL;

V_{ST}: Sample total volume, 1 mL;

Note:

1. Prepare Reagent IV before use and protect from light.
2. The reaction of proline and hydroxyproline with ninhydrin has no absorption peak at 570 nm. Therefore, the determination result at 570 nm does not contain these two amino acids.
3. When ΔA_T is more than 1, it is recommended to dilute the sample with Reagent I before determination.

Experimental example:

1. Take 0.15g soil sample to 1ml Reagent I, and operate as the procedure after taking the supernatant, A_T=0.304, A_S=0.603, A_B=0.064, $\Delta A_T=A_T-A_B$=0.304-0.064=0.240, $\Delta As=As-A_B$= 0.603-0.064=0.539, calculate content by sample weight:
   AA（μmol/g weight)= $6\times\Delta A_T\div\Delta As\div W=6\times0.240\div0.539\div0.15$=17.811 μmol/g weight.

References:

[1] Wang X, Cui X Y, Guo Y F A study on free amino acid in different forest types soil of cold-temperate forest region[J]. Journal of Nanjing Forestry University (Natural Science Edition), 2016, 59(04): 42-48.

[2] Wang B, Jiang Y Y, Jiao J G, et al. Effects of earthworm on constituent and amount of

amino acid

in soil[J]. Acta Ecologica Sinica, 2015,35(14):4816-4823.

[3] Zhang X J, Wang W Y, Li W Q, et al. Dynamics of soil dissolved organic nitrogen in alpine meadow[J]. Journal of Lanzhou University: Natural Sciences, 2016, 52(5):623-627.

Related Products:

BC1530/BC1535	Urea Nitrogen Content Assay Kit
BC1500/BC1505	Plant Nitrate Nitrogen Content Assay Kit
BC1520/BC1525	Plant Ammonium Nitrogen Content Assay Kit

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