Soil glutaminase (S-GLS) Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: NA0375

Size:100T/48S

Components:

Reagent I: 30mL×1, storage at 4℃.

Reagent II: Powder×1, storage at 4℃. Dissolve with 15mL of distilled water before use.

Reagent IIIA: 0.4mL×1, storage at 4℃.

Reagent IIIB: 1.6mL×1, storage at 4℃. Before use, pour reagent IIIA into reagent IIIB and mix before use (IIIA: IIIB = 1: 4 (V:V)).

Reagent IV: 2mL×1, storage at RT.

Standard: 1mL×1, storage at 4℃. 10μmnol/mL NH_4^+ standard solution.

Product Description:

S-GLS (EC3.5.1.2) exists in some bacteria and plant roots, catalyzes the hydrolysis of glutamine to glutamic acid and ammonia. It has important regulatory effects on nitrogen metabolism, especially the regulation of free ammonia content and urea metabolism.

S-GLS catalyzes the hydrolysis of glutamine to L-glutamic acid and ammonia. The rate of ammonia increase can be calculated by the indophenol blue colorimetric method, and its enzyme activity can be calculated.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ Microplate reader, adjustable transferpettor, balance, mortar/homogenizer, centrifuge, micro glass cuvette/ 96-well plate, sieve (30-50 mesh, or smaller), toluene, ice and distilled water.

Sample preparation:

Fresh soil samples are naturally air-dried or oven dried at 37℃ and passed the sieve (30-50 mesh).

Procedure:

1. Preheat spectrophotometer/ microplate reader for 30min, adjust the wavelength to 630 nm and set the counter to zero with distilled water.
2. Dilute the standard solution 64 times with distilled water to prepare 0.156 μmol/mL standard solution
3. Add reagent to a 1.5mL EP tube:

Reagent name	Test tube (At)	Control tube (Ac)	Standard tube (As)	Blank tube (Ab)

Sample (g)	0.05	0.05		
Toluene (µL)	25	25		
React 10min at room temperature.				
Reagent I (µL)	275	275		
Reagent II (µL)	200			
Distilled water (µL)		200		80
Mix well and incubate at 37 ° C for 1 hour. Centrifuge at 10,000 g for 10 min at room temperature and take the supernatant.				
Supernatant	80	80		
Standard solution			80	
Reagent III（µL）	16	16	16	16
Reagent IV（µL）	12	12	12	12
Distilled water	92	92	92	92

Mix well and react at room temperature for 30min. After cooling, the absorbance at the wavelength of 630nm, and record them as At, Ac, As, and Ab, and calculate $\Delta A = At-Ac$, $\Delta As = As-Ab$.

Calculation:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme the production of glutamine into 1µmol NH_4^+ per day every gram soil catalyzes in the reaction system.

S-GLS (U/g) = $\Delta A \div (\Delta As \div Cs) \times Vr \div T \div W = 1.872 \times \Delta A \div \Delta As \div W$.

Cs: standard concentration，0.156µmoL/mL；

T：reaction time, 1/24 d;

Vr: reaction volume, 0.5 mL;

W: soil weight, g;

Note:

1. When the A is greater than 0.8, it is recommended to further dilute the supernatant and measure.

2. If the supernatant still contains a small amount of impurities after centrifugation, the supernatant can be removed again by 10,000g centrifuge at room temperature for 10min.

3. Use reagent3 as soon as possible after configuration. If discoloration is found, do not use it again.

Experimental examples:

1. Take two tubes of 0.1g clover soil and mark them as test tube and control tube respectively, and follow the measurement procedure. After determination with 96-well plate, calculate $\Delta A = At-Ac = 0.8-0.175 = 0.625$，$\Delta As = As-Ab = 0.268-0.044 = 0.224$. The enzyme activity is calculated according to the sample mass.

S-GLS (U/g) = $1.872 \times \Delta A \div \Delta As \div W = 104.46$ U/g.

2. Take two tubes of 0.1g woodland and mark them as test tube and control tube respectively, and follow the measurement procedure. After determination with 96-well plate, calculate $\Delta A = At-Ac = 0.466-0.164 = 0.302$，$\Delta As = As-Ab = 0.268-0.044 = 0.224$. The enzyme activity is calculated according to the sample mass.

S-GLS (U/g) $= 1.872 \times \Delta A \div \Delta As \div W = 50.477$ U/g.

Related products:

NA0644/NA0402   Soil Nitrate Reductase(S-NR) Activity Assay Kit

NA0361/NA0360   Soil β-1,4-Glucanase Activity Assay Kit

NA0371/NA0362   Soil Leucine Arylamidase(S-LAP) Activity Assay Kit

NA0850/NA0608   Soil Saccharase(S-SC) Activity Assay Kit