Soil glutaminase (S-GLS) Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: BC3975

Size:100T/48S

Components:

Reagent I: 30mL×1, storage at 4℃.

Reagent II: Powder×2, storage at 4℃. Dissolve with 6 mL of distilled water before use,unused reagents can be stored at 4℃ for 1 week.

Reagent IIIA: 0.4 mL×1, storage at 4℃.

Reagent IIIB: 1.6 mL×1, storage at 4℃.

Preparation of Reagent III: 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℃. 10μmol/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:

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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 ΔA = At-Ac, ΔAs = 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) = ΔA÷ (ΔAs ÷Cs)× Vr ÷ T÷W=1.872×ΔA÷ΔAs÷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 ΔA = At-Ac =0.8-0.175=0.625，ΔAs = As-Ab =0.268-0.044=0.224. The enzyme activity is calculated

according to the sample mass.

S-GLS (U/g) = 1.872×ΔA÷ΔAs÷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 ΔA = At-Ac =0.466-0.164=0.302， ΔAs = As-Ab =0.268-0.044=0.224. The enzyme activity is calculated according to the sample mass.

   S-GLS (U/g) =1.872×ΔA÷ΔAs÷W =50.477 U/g.

Related products:

BC3100/BC3105	Soil Nitrate Reductase(S-NR) Activity Assay Kit
BC4030/BC4035	Soil β-1,4-Glucanase Activity Assay Kit
BC4020/BC4025	Soil Leucine Arylamidase(S-LAP) Activity Assay Kit
BC0240/BC0245	Soil Saccharase(S-SC) Activity Assay Kit

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