Soil carbonic anhydrase (S-CA) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC5505

Size: 100T/48S

Components:

Reagent I: Liquid 40 mL×1, Storage at 2-8℃;

Reagent II: Powder×2, Storage at 2-8℃. Before use, take a bottle of Reagent II and add 650μL acetone to dissolve the powder thoroughly, then add 6.7mL distilled water. Unused reagents can be stored in aliquots at -20°C for 1 weeks, avoiding repeated freezing and thawing;

Standard solution: Liquid 1 mL×1, Storage at 2-8℃; 5μmol/mL phenol standard solution. Before use, 50μL of 5μmol/mL phenol standard solution was taken into the reagent bottle, and 750μL distilled water was added to mix thoroughly to form 0.3125μmol /mL phenol standard solution.

Product Description:

Carbonic Anhydrase (CA, EC4.2.1.1) is a metal enzyme with Zn^{2+} as the active center, which can be used to efficiently catalyze reversible hydration reaction of CO_2: $CO_2+H_20 \leftrightharpoons HCO^{3-}+H^+$, with a catalytic rate up to 10^7 times that of natural conditions, which is one of the fastest enzymes known so far.

Carbonic anhydrase can catalyze the reaction of acetic acid to nitrophenyl ester to p-nitrophenol. The activity of carbonic anhydrase can be reflected by detecting the increase rate of absorption value at 405nm.

Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, water-bath/ constant temperature incubator, adjustable pipette, micro quartz cuvette/96 well flat-bottom UV plate, 30-50 mesh sieve, Toluene, ice and distilled water.

Protocol

I. Preparation:

Fresh soil sample natural air dry or 37℃ oven air dry, 30~50 mesh sieve.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 405 nm, set the counter to zero with distilled water.
2. Preheat Reagent I in 37℃ (mammal) or 25℃ (other species) for 15 minutes.
3. Standard tube measurement

1) Standard tube measurement: 80μL standard solution and 320μL Reagent I were added into 1.5mL EP tube, thoroughly mixed, and the light absorption value at 405nm was measured in micro quartz cuvette/96 well flat-bottom UV plate, which was referred to as A_S.

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2) Standard blank tube measurement: 80μL distilled water and 320μL Reagent I were added into 1.5mL EP tube. After full mixing, the absorbance value at 405nm was measured in micro quartz cuvette/96 well flat-bottom UV plate, which was recorded as A_{SB}.
3) calculation $\Delta A_S = A_S - A_{SB}$. (Standard tubes and standard blank tubes only need to be done 1-2 times.)
4. Operation table: (Add to 1.5mLEP tube)

Reagent Name (μL)	Test tube (A_T)	Control tube (A_C)
Sample	0.1g	0.1g
Toluene	20	20
Fully shake the soil to make it moist, and leave it at room temperature for 15minutes		
Reagent I	300	300
-	-	Boil for 10min and cool with ice water
Reagent II	80	80
After reaction at 37℃ for 5minutes, it was immediately placed in an ice bath, and then centrifuged at 4℃ for 15000g for 10minutes. 0.2mL supernatant was absorbed into micro quartz cuvette/96 well flat-bottom UV plate to determine the light absorption value at 405nm, which was denoted as A_T and A_C. Calculate $\Delta A = A_T - A_C$. (Each measuring tube corresponds to a pair of tubes).		

III. Calculation of soil CA activity:

Sample weight:

Unit definition: The catalytic production of 1μmol p-nitrophenol per g of soil per minute at 37℃ was defined as a unit of enzyme activity.

$$S\text{-}CA\ (U/g\ weight) = C_S \times \Delta A \div \Delta A_S \times V_S \div W \div T \times F = 0.005 \times \Delta A \div \Delta A_S \div W \times F$$

C_S: Standard concentration, 0.3125μmol/mL;
V_S: Standard solution volume added to the reaction system, 0.08mL;
T: Reaction time, 5 minutes;
W: Sample weight, g;
F: Sample dilution ratio.

Note:

1. If A>1.5 or ΔA>0.8, the sample size can be reduced or the enzymatic reaction time at 37℃ can be shortened; When ΔA<0.02, the sample size can be increased or the enzymatic reaction time at 37℃ can be prolonged. Note that the calculation formula is changed simultaneously during calculation.
2. If the supernatant to be measured is still cloudy after centrifugation, try to increase the centrifugal speed or extend the time, for example, 20000g, centrifugation at 4℃ for 10min

Experimental example:

1. Take 0.1016g soil sample No. 16, operate according to the measurement steps, use 1mL glass cuvette to measure $\Delta A=A_T-A_C=0.353-0.227=0.126$, $\Delta A_S=A_S-A_{SB}=0.65-0.046=0.604$, put into the formula to calculate:

   S-CA activity (U/g weight) $=0.005\times\Delta A\div\Delta As\div W\times F=0.010$ U/g weight
2. Take 0.1056g soil sample No. 62, operate according to the measurement steps, use 1mL glass cuvette to measure $\Delta A=A_T-A_C=0.577-0.271=0.306$, $\Delta A_S=A_S-A_{SB}=0.650-0.046=0.604$, put into the formula to calculate:

   S-CA activity (U/g weight) $=0.005\times\Delta A\div\Delta As\div W\times F=0.024$ U/g weight

Recent Product Citations：

[1] Li W, Yu L J, Yuan D X, et al. A study of the activity and ecological significance of carbonic anhydrase from soil and its microbes from different karst ecosystems of Southwest China[J]. Plant and Soil, 2005, 272(1):133-141.

Related Products：

BC0150/BC0155	Soil Cellulase (S-CL) Activity Assay Kit
BC0160/BC0165	Soil β-glucosidase (S-β-GC) Activity Assay Kit

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