EZMeta™ Soil Catalase (S-CAT) Colorimetric Activity Kit

Cat #: D-AKC4050

Size: 48T / 96T

Storage: Stored at 4°C for 12 months

Product Information

Applicable samples: Soil sample

Assay Principle

Catalase (CAT, EC 1.11.1.6), an enzyme scavenger, is a conjugated enzyme with iron porphyrin as the auxiliary group, which can promote the decomposition of H_2O_2 into molecular oxygen and water. Soil catalase (S-CAT) is an important enzyme in soil microbial metabolism and plays an important role in H_2O_2 scavenging system. H_2O_2 has a characteristic absorption peak at 240 nm. The activity of S-CAT can be reflected by measuring the change of absorbance of soil extract solution at this wavelength before and after the reaction.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	300 µL	600 µL	4°C
Reagent II	1	1	4°C
Reagent III	3 mL	6 mL	4°C

Materials Required but Not Supplied

·Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 240 nm

·96-well UV plate or micro quartz cuvette, precision pipettes, disposable pipette tips

·Thermostat water bath, constant temperature shaker, centrifuge, 30-50 mesh sieve

·Deionized water

Reagent Preparation

Reagent I: Prepare before use, add 29.7 mL deionized water for 48 T and 59.4 mL deionized water for 96 T to fully dissolve. Store at 4°C.

Reagent II: Prepare before use, add 1 mL deionized water for 48 T and 2 mL deionized water for 96 T to fully dissolve. Store at 4°C (If there is crystallization, use it after dissolving in a 60°C-90°C water bath).

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Sample Preparation

Note: Fresh samples are recommended. If the experiment is not carried out immediately, the samples can be stored at -80°C for several weeks. During the determination, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

Soil sample: naturally dried fresh soil samples or air drying in oven at 37°C, pass through a 30-50 mesh sieve.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 240 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Operation table (The following were operated in 1.5 mL EP tube):

Reagent	Test Well	No matrix Well	Soilless Well
Air dried soil sample (g)	0.03	0.03	0
Reagent I (μL)	260	0	260
Deionized Water (μL)	0	260	0
Shaking culture at 25°C for 20 min			
Reagent II (μL)	10	10	10
Mix well, centrifuge at 8,000 g for 5 min at 25°C, and take 180 μL supernatant to 96-well UV plate or micro quartz cuvette			
Reagent III (μL)	20	20	20

3. Mix well, measure the absorbance of each tube at 240 nm, and record as A_{Test}, $A_{No\ matrix}$ and $A_{Soilless}$.

Note: Soilless well only need to measure 1 time. No matrix well is necessary for each test well. In order to guarantee the accuracy of experimental results, pre-experiments are suggested for 2-3 samples with potential significant difference.

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.

A. 96-well UV plates calculation formula as below.

Definition of unit: the degradation of 1 μmol H_2O_2 catalyzed by each g of air dried soil sample per day is defined as one unit of enzyme activity.

S-CAT(U/g soil sample)=$[(A_{Soilless}-A_{Test}+A_{No\ matrix})\times V_{Total\ volume}\div(\varepsilon\times d)\times 10^6]\div W\div T=\mathbf{33\times(A_{Soilless}-A_{Test}+A_{No\ matrix})}$

$V_{Total\ volume}$: total volume of reaction system, 3×10^{-4} L; ε: molar extinction coefficient of hydrogen peroxide, 4.36×10^4 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; T: reaction time, 20 min=1/72 d; W: mass of soil sample, 0.03 g.

B. Microquartz cuvette calculation formula as below.

Definition of unit: the degradation of 1 μmol H_2O_2 catalyzed by each g of air dried soil sample per day is defined as one unit of enzyme activity.

S-CAT(U/g soil sample)=[($A_{Soilless}$-A_{Test}+$A_{No\ matrix}$)×$V_{Total\ volume}$÷(ε×d)×10^6]÷W÷T=**16.5×($A_{Soilless}$-A_{Test}+$A_{No\ matrix}$)**

$V_{Total\ volume}$: total volume of reaction system, 3×10^{-4} L; ε: molar extinction coefficient of hydrogen peroxide, 4.36×10^4 L/mol/cm; d: cuvette optical diameter, 1 cm; T: reaction time, 20 min=1/72 d; W: mass of soil sample, 0.03 g.

Typical Data

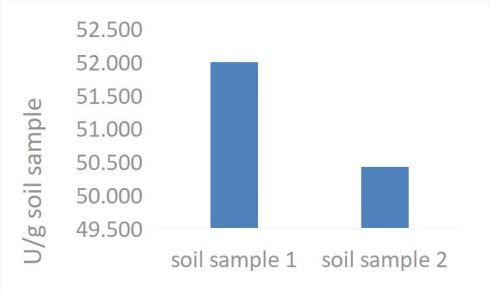

Figure 1. Determination of catalase activity in soil by this assay kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.