

Soil Neutral Protease Activity Assay Kit - Spectrophotometric Method

Product Code

67032

Product Introduction

Soil protease participates in the transformation of amino acids, proteins, and other protein-containing nitrogenous organic compounds in soil. Its hydrolysates are one of the nitrogen sources for higher plants.

Soil neutral protease activity (S-NPT) catalyzes protein hydrolysis in a neutral environment and is related to soil organic matter content, nitrogen, and other soil properties. Under neutral conditions, S-NPT hydrolyzes casein to produce tyrosine. Under alkaline conditions, tyrosine reduces phosphomolybdic acid compounds to form tungsten blue, which has a characteristic absorption peak at 680 nm.

Package List

Specification	Item Code	Component	Quantity
50T	67032.1	Reagent One	1 bottle
50T	67032.2	Reagent Two	1 bottle
50T	67032.3	Reagent Three	1 bottle
50T	67032.4	Reagent Four	1 bottle
50T	67032.5	Reagent Five	1 bottle
50T	67032.6	Reagent Six	1 bottle
50T	67032.7	Reagent Seven	1 bottle
50T	67032.m	Manual	1 copy

Quality Standards and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent One	Not specified	Not specified
Reagent Two	Not specified	Not specified
Reagent Three	Not specified	Not specified
Reagent Four	Not specified	Not specified
Reagent Five	Not specified	Not specified
Reagent Six	Not specified	Not specified
Reagent Seven	Not specified	Not specified

Shipping and Storage

Shipping	Shipped with ice packs.
Storage	Store at 2-8°C, protected from light.
Shelf Life	180 days.

Instructions for Use

1. Sample Processing

Naturally air-dry fresh soil samples or dry them in a 37°C oven. Pass the dried samples through a 30-50 mesh sieve.

2. Reagent Preparation

- **Reagent II:** Before use, add 6 mL distilled water and dissolve completely. Store unused reagent at 4°C.
- **Reagent III:** Before use, add 2 mL Reagent VII and heat in a boiling water bath to dissolve. Then add 8 mL Reagent I and mix thoroughly for later use. Store unused reagent at 4°C.
- **Reagent IV:** Before use, add 40 mL distilled water and dissolve completely. Store unused reagent at 4°C.
- **Reagent VI:** 0.05 mg/mL standard tyrosine solution. Store at 4°C.

3. Assay Procedure

1. Preheat the spectrophotometer for more than 30 minutes.
2. Set the wavelength to 680 nm.
3. Zero the instrument with distilled water.

3.1 Enzymatic Reaction

Component or Step	Assay Tube	Control Tube
Air-dried soil sample	0.04 g	0.04 g
Reagent I	300 µL	-
Reagent III	-	300 µL
Incubation	Mix thoroughly. Incubate in a 40°C water bath for 30 minutes. Shake 5-6 times to allow the soil sample to fully contact the reaction solution.	
Reagent II	100 µL	100 µL
Centrifugation	Mix thoroughly. Centrifuge at 8000g and 25°C for 10 minutes, then collect the supernatant.	

3.2 Color Development and Measurement

Component or Step	Assay Tube	Control Tube	Standard Tube
Supernatant	150 µL	150 µL	-
Reagent VI	-	-	150 µL
Reagent IV	700 µL	700 µL	700 µL
Reagent V	150 µL	150 µL	150 µL
Incubation and centrifugation	Mix well. Incubate in a 40°C water bath for 20 minutes. Centrifuge at 8000g and 25°C for 10 minutes.		
Measurement	Take 1 mL of supernatant and read the absorbance of each tube at 680 nm.		

The standard tube only needs to be measured once. Prepare one control tube for each assay tube.

Calculation

Unit definition: The amount of enzyme activity that produces 1 mg tyrosine per day from each g of soil sample is defined as one S-NPT activity unit.

$$\text{S-NPT (mg/d/g soil sample)} = \frac{C \text{ standard} \times (A \text{ assay tube} - A \text{ control tube})}{A \text{ standard tube} \times V \text{ total reaction} \div W \div T} = 24 \times \frac{(A \text{ assay tube} - A \text{ control tube})}{A \text{ standard tube}}$$

Symbol	Meaning	Value
C standard tube	Standard tube concentration	0.05 mg/mL
V total reaction	Total volume of the reaction system	0.4 mL
T	Reaction time	30 min = 1/48 d
W	Sample mass	0.04 g

Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for a preliminary test.

2. Instruments and supplies required but not provided: visible spectrophotometer, water bath, magnetic stirrer, adjustable pipette, 1 mL glass cuvette, and distilled water.
3. This 50T kit can test 24 samples.

Visual Reference