Soil Neutral Protease Activity Assay Kit

Product Code: 67043

Soil Neutral Protease Activity Assay Kit - Microplate Method

Product Information

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

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 Contents

Product Code	Component	Quantity
67043.1	Reagent I	1 bottle
67043.2	Reagent II	1 bottle
67043.3	Reagent III	1 bottle
67043.4	Reagent IV	1 bottle
67043.5	Reagent V	1 bottle
67043.6	Reagent VI	1 bottle
67043.7	Reagent VII	1 bottle
67043.m	Instruction manual	1 copy

Quality and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Reagent III	--	--
Reagent IV	--	--
Reagent V	--	--
Reagent VI	--	--
Reagent VII	--	--

Transportation and Storage

Condition	Requirement
Transportation	Transport with ice packs.
Storage	Store at 2-8°C, protected from light.
Shelf life	180 days

Materials Required but Not Supplied

- Microplate reader
- Water bath
- Magnetic stirrer
- Adjustable pipette

- 96-well microplate
- Double-distilled water

Sample Processing

Air-dry fresh soil samples naturally or dry them in an oven at 37°C. Pass the dried samples through a 30-50 mesh sieve before testing.

Reagent Preparation

- **Reagent II:** Before use, add 6 mL distilled water and dissolve thoroughly. 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, mix thoroughly, and set aside for use. Store unused reagent at 4°C.
- **Reagent IV:** Before use, add 30 mL distilled water and dissolve thoroughly. Store unused reagent at 4°C.
- **Reagent VI:** 0.05 mg/mL standard tyrosine solution. Store at 4°C.

Assay Procedure

Instrument Setup

Preheat the microplate reader for at least 30 minutes. Set the wavelength to 680 nm and zero the instrument with distilled water.

Enzymatic Reaction

Component	Assay Tube	Control Tube
Air-dried soil sample (g)	0.02	0.02
Reagent I (μL)	150	
Reagent III (μL)	150	

1. Mix thoroughly and incubate in a 40°C water bath for 30 minutes.
2. Shake 5-6 times during incubation so the soil sample fully contacts the reaction solution.
3. Add 50 μL Reagent II to both the assay tube and control tube.
4. Mix thoroughly, then centrifuge at 8000 g and 25°C for 10 minutes.
5. Collect the supernatant for color development.

Color Development and Measurement

Component	Assay Tube	Control Tube	Standard Tube
Supernatant (μL)	60	60	
Reagent VI (μL)	60		
Reagent IV (μL)	280	280	280
Reagent V (μL)	60	60	60

1. Mix well and incubate in a 40°C water bath for 20 minutes.
2. Centrifuge at 8000 g and 25°C for 10 minutes.
3. Transfer 200 μL supernatant to a 96-well microplate.
4. Read the absorbance value A of each tube at 680 nm.

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

Calculation

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

$$\text{S-NPT (mg/d/g soil sample)} = C_{standard} \times (A_{assay\ tube} - A_{control\ tube}) \div A_{standard\ tube} \times V_{total\ reaction} \div W \div T$$

$$\text{S-NPT (mg/d/g soil sample)} = 24 \times (A_{assay\ tube} - A_{control\ tube}) \div A_{standard\ tube}$$

Symbol	Description	Value
$C_{standard}$	Standard tube concentration	0.05 mg/mL
$V_{total\ reaction}$	Total reaction system volume	0.4 mL
T	Reaction time	30 min = 1/48 d
W	Sample mass	0.02 g

Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for a preliminary test.
2. This 100T kit can test 48 samples.

Visual Reference