

Soil Neutral Phosphatase (S-NP) Activity Assay Kit - Microplate Method**Product Introduction**

Soil phosphatase catalyzes the mineralization of soil organic phosphorus. Its activity directly affects the decomposition, transformation, and bioavailability of organic phosphorus in soil, and it is used as an indicator for evaluating the direction and intensity of soil phosphorus biotransformation.

Phosphatase activity is significantly affected by soil carbon and nitrogen content, available phosphorus content, and pH. According to the optimum pH range, soil phosphatases are generally divided into neutral, acidic, and alkaline types.

In a neutral environment, S-NP catalyzes the hydrolysis of disodium phenyl phosphate to produce phenol and disodium hydrogen phosphate. S-NP activity is calculated by measuring the amount of phenol produced.

Actual readings may vary under different testing conditions and with different instruments. Reference data or effect diagrams are for reference only.

Product Packing List

| Size | Code | Component | Quantity |
|------|---------|--------------------|----------|
| 100T | 67041.1 | Reagent I | 1 bottle |
| 100T | 67041.2 | Reagent II | 1 bottle |
| 100T | 67041.3 | Reagent III | 1 bottle |
| 100T | 67041.4 | Reagent IV | 2 tubes |
| 100T | 67041.5 | Standard | 1 bottle |
| 100T | 67041.m | Instruction Manual | 1 copy |

Quality Standards and Safety Instructions

| Raw Material or Packaging Name | Quality Standard | Main Toxicity |
|--------------------------------|------------------|---------------|
| Reagent I | -- | -- |
| Reagent II | -- | -- |
| Reagent III | -- | -- |
| Reagent IV | -- | -- |
| Standard | -- | -- |

Shipping and Storage Conditions

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

Instructions for Use**1. Reagent Preparation**

1. Add 100 mL distilled water to Reagent II before use and dissolve thoroughly.
2. Before use, add 576 μ L anhydrous ethanol, self-prepared, and 24 μ L distilled water to each vial of Reagent IV. Dissolve thoroughly.

Do not use Reagent IV after it turns brown.

2. Catalytic Reaction

1. Weigh approximately 0.1 g of air-dried, well-mixed soil.
2. Add 0.05 mL toluene, self-prepared, and gently shake for 15 min.
3. Add 0.4 mL Reagent I and mix well.
4. Place the mixture in a 37°C constant-temperature incubator and start timing.
5. Carry out the catalytic reaction for 24 h.
6. When the reaction time is reached, quickly add 1 mL Reagent II and mix thoroughly to terminate the enzyme-catalyzed reaction.
7. Centrifuge at 10000 rpm at room temperature for 10 min.
8. Collect the supernatant and place it on ice for testing.

3. Color Development Reaction

1. Preheat the microplate reader for 30 min or more, and set the wavelength to 660 nm.
2. Blank well: add 10 µL distilled water, 20 µL Reagent III, and 4 µL Reagent IV to a microplate well. Mix thoroughly. After color development, add 166 µL distilled water, mix well, and let stand at room temperature for 30 min. Measure the absorbance at 660 nm and record as A_{blank} .
3. Standard well: add 10 µL standard solution, 20 µL Reagent III, and 4 µL Reagent IV to a microplate well. Mix thoroughly. After color development, add 166 µL distilled water, mix well, and let stand at room temperature for 30 min. Measure the absorbance at 660 nm and record as A_{standard} .
4. Measurement well: add 10 µL supernatant, 20 µL Reagent III, and 4 µL Reagent IV to a microplate well. Mix thoroughly. After color development, add 166 µL distilled water, mix well, and let stand at room temperature for 30 min. Measure the absorbance at 660 nm and record as $A_{\text{measurement}}$.

The blank well and standard well only need to be measured once.

4. S-NP Activity Calculation

Activity unit definition: at 37°C, the release of 1 µmol phenol per gram of soil per day is defined as one enzyme activity unit.

$$\text{S-NP (U/g)} = [C_{\text{standard}} \times (A_{\text{measurement}} - A_{\text{blank}}) \div (A_{\text{standard}} - A_{\text{blank}})] \times V_{\text{total}} \div W \div T$$

$$\text{S-NP (U/g)} = 0.725 \times (A_{\text{assay}} - A_{\text{blank}}) \div (A_{\text{standard}} - A_{\text{blank}}) \div W$$

| | |
|-----------------------|---|
| C_{standard} | 0.5 µmol/mL |
| V_{total} | Total volume of the catalytic system, 1.45 mL |
| W | Soil sample mass, g |
| T | Catalytic reaction time, 24 h = 1 d |

Precautions

1. This 100T product can test 96 samples.
2. Anhydrous ethanol and toluene must be prepared separately.
3. Reagent IV cannot be used after it turns brown.

Visual Reference