# Soil Neutral Phosphatase(S-NP) Activity Assay Kit

Product Code: 67049

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**Method:** Spectrophotometry

## 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, making it 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 optimal pH range, soil phosphatases are generally divided into neutral, acidic, and alkaline types.

In a neutral environment, soil neutral phosphatase 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.

## Product Package List

| Specification | Component Code | Component | Quantity |
|---|---|---|---|
| 50T | BR5000092.1 | Reagent I | 1 bottle |
| 50T | BR5000092.2 | Reagent II | 1 bottle |
| 50T | BR5000092.3 | Reagent III | 1 bottle |
| 50T | BR5000092.4 | Reagent IV | 1 bottle |
| 50T | BR5000092.5 | Standard | 1 bottle |
| 50T | BR5000092.m | Manual | 1 copy |

## Quality Standards and Safety Information

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

## Transportation and Storage

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

## Product Instructions

### 1. Reagent Preparation

1. Before use, add 60 mL distilled water to Reagent II and dissolve thoroughly.
2. Immediately before use, add 1152 μL absolute ethanol, self-prepared, and 48 μL distilled water to Reagent IV and 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 shake gently 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. Allow the catalytic reaction to proceed for 24 h.
6. When the reaction time is reached, quickly add 1 mL Reagent II and mix thoroughly to stop the enzyme-catalyzed reaction.
7. Centrifuge at 10000 rpm at room temperature for 10 min.
8. Place the supernatant on ice for testing.

### 3. Color Reaction

1. Preheat the spectrophotometer for at least 30 min and set the wavelength to 660 nm. Use distilled water to zero the instrument.
2. Blank tube: In a 1 mL glass cuvette, add 50 μL distilled water, 100 μL Reagent III, and 20 μL Reagent IV. Mix thoroughly. After color development, add 830 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 tube: In a 1 mL glass cuvette, add 50 μL standard solution, 100 μL Reagent III, and 20 μL Reagent IV. Mix thoroughly. After color development, add 830 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 tube: In a 1 mL glass cuvette, add 50 μL supernatant, 100 μL Reagent III, and 20 μL Reagent IV. Mix thoroughly. After color development, add 830 μL distilled water, mix well, and let stand at room temperature for 30 min. Measure the absorbance at 660 nm and record as $A_{measurement}$.

> The blank tube and standard tube need to be measured only 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.

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

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

| | |
|---|---|
| $C_{standard}$ | 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 50T kit can test 48 samples.
2. Absolute ethanol and toluene must be prepared separately.
3. Reagent IV cannot be used after it turns brown.

## Visual Reference