

## Soil Chitinase Activity Assay Kit - Microplate Method

### Product Information

**Product code:** 67053

Chitin is mainly found in the shells of crustaceans such as shrimp and crabs, in insects, in animal soft tissues such as cuttlefish cartilage, and in fungal cell walls. Chitinase (EC 3.2.1.14) catalyzes the hydrolysis of chitin and plays a role in resistance to fungal infection, making it an important research target for antifungal disease control.

Chitinase hydrolyzes chitin to produce N-acetylglucosamine, which reacts with p-dimethylaminobenzaldehyde to form a red compound. The increase in absorbance reflects chitinase activity.

### Package Contents

| Size | Code    | Component    | Quantity |
|------|---------|--------------|----------|
| 100T | 67053.1 | Reagent I    | 1 bottle |
| 100T | 67053.2 | Reagent II   | 1 bottle |
| 100T | 67053.3 | Reagent III  | 1 bottle |
| 100T | 67053.m | Instructions | 1 copy   |

### Quality and Safety Information

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

### Transportation and Storage

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

### Instructions for Use

#### 1. Sample Processing

Air-dry fresh soil samples and pass them through a 30-50 mesh sieve.

#### 2. Assay Procedure

| Step                | Control Tube                                    | Assay Tube |
|---------------------|---|------------|
| Add soil sample     | 0.1 g   | 0.1 g      |
| Add toluene         | 15 uL   | 15 uL      |
| Mix and stand       | Mix well and stand at 25 degrees C for 15 min.  |            |
| Add distilled water | 100 uL  | --         |
| Add Reagent I       | --  | 100 uL     |
| Incubate            | Mix well and incubate at 37 degrees C for 24 h. |            |

|                     |   |        |
|---------------------|---|--------|
| Add distilled water | 200 uL  | 200 uL |
| Centrifuge          | Centrifuge at 4000 rpm, 4 degrees C for 30 min. Transfer 300 uL of supernatant into a new tube.   |        |
| Add Reagent II      | 20 uL   | 20 uL  |
| Heat                | Mix well and place in a boiling water bath for 5 min.   |        |
| Add Reagent III     | 600 uL  | 600 uL |
| Color development   | Mix well and develop color at 37 degrees C for 20 min.  |        |
| Measurement         | Transfer 200 uL to a 96-well plate. Zero with distilled water, measure absorbance at 544 nm, and record as A control tube and A assay tube. |        |

Calculate the absorbance difference as follows:

$$\Delta A = A_{\text{assay tube}} - A_{\text{control tube}}$$

### 3. Calculation

Standard curve:

$$y = 2.6357x - 0.0007, R^2 = 0.9989$$

In the formula, x is the standard concentration in ug/mL, and y is the absorbance value Delta A.

Definition of enzyme activity: at 37 degrees C, the amount of enzyme that decomposes chitin in each gram of soil per day to produce 1 ug N-acetylglucosamine is defined as one unit of enzyme activity.

$$\text{Soil chitinase activity (ug/d/g soil sample)} = (\Delta A + 0.0007) / 2.6357 \times V_{\text{total reaction}} / W / T \times 1000 = 1195 \times (\Delta A + 0.0007)$$

| Symbol           | Description                                   |
|------------------|---|
| V total reaction | Total volume of the reaction system: 0.315 mL |
| W                | Sample mass: 0.1 g                            |
| T                | Reaction time: 1 d                            |
| 1000             | Conversion factor: 1 mg/mL = 1000 ug/mL       |

### Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for a preliminary test.
2. Prepare the following instruments and supplies before use: balance, water bath, centrifuge, shaker, microplate reader, 96-well plate, toluene, and distilled water.
3. Mix Reagent I thoroughly before use.
4. This 100T kit can test 48 samples.