# Chitinase Assay Kit

**Catalog No.:** abx298854

**Size:** 96 tests

**Detection Range:** 3.12 U/ml – 250 U/ml

**Sensitivity:** 3.12 U/ml

**Storage:** Store all components at 4°C.

**Application:** For detection and quantification of Chitinase activity in tissue homogenates and cell samples.

## Introduction

Chitinases are a group of enzymes which catalyze the breakdown of chitin, a long chain polymer of acetylglucosamine. These enzymes are most abundant in the cells of insects, crustaceans, mollusks, and fungi, which use chitin chains to provide structure to various parts of their anatomy. The ability of chitinases to degrade the cell walls of fungi and the exoskeleton of insects is of particular interest to researchers in both the agricultural and medical fields, where fungal and insect-vectored diseases represent a growing threat.

Abbexa's Chitinase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Chitinase activity. In the presence of chitin, Chitinase catalyzes the production of compounds with an absorbance maximum at 585 nm. The intensity of the color is proportional to the Chitinase activity, which can then be calculated over time.

## Kit components

1. 96-well microplate
2. Assay Buffer: 15 ml
3. Extraction Solution: 30 ml
4. Matrix Solution: 12 ml
5. Saline Solution: 5 ml
6. Chromogenic Reagent: 2 vials
7. Chromogenic Reagent Diluent: 70 ml
8. Standard: 1 vial
9. Plate sealer: 2

## Materials required but not provided

1. Microplate reader (585 nm)
2. PBS (0.01 M, pH 7.4)
3. Pipette and pipette tips
4. 1.5 ml microcentrifuge tubes
5. Centrifuge
6. Water bath
7. Incubator

## Protocol

### A. Preparation of samples and reagents

#### 1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh out at least 50 mg of tissue, and wash in ice-cold PBS (0.01 M, pH 7.4). Add the tissue into Extraction Solution in a ratio of 1 : 9 weight (mg) to volume (µl) (i.e. for 50 mg of tissue, add into 450 µl of Extraction Solution). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath at 4°C. Collect the resulting homogenate, and centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully remove the supernatant, keep on ice, and assay immediately.

  **Note:** To calculate Chitinase activity in tissue homogenates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (**abx097193**).

- **Cell Lysates:** Collect at least $1\times10^6$ cells, and wash in ice-cold PBS (0.01 M, pH 7.4). Add the cells into Extraction Solution in a ratio of 1 : 200 cells ($1\times10^6$) to volume (µl) (i.e. for $1\times10^6$ fungal cells, add into 200 µl of Extraction Solution). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath at 4°C. Collect the resulting homogenate, and centrifuge at 10,000 × g for 20 minutes at 4°C. Carefully remove the supernatant, keep on ice, and assay immediately.

  **Note:** To calculate Chitinase activity in cell lysates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (**abx097193**).

**Note:**

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

## 2. Reagents

- **Working Chromogenic Reagent:** Dissolve one vial of Chromogenic Reagent in 32 ml of Chromogenic Reagent Diluent. Mix well, until fully dissolved. The reconstituted Working Chromogenic Reagent can be stored for up to 4 weeks at 4°C in the dark.
- **Stock (5 mg/ml) Standard Solution:** Dissolve one vial of Standard in 1 ml of Assay Buffer. Mix well, until fully dissolved. The reconstituted Stock (5 mg/ml) Standard Solution can be stored for up to 4 weeks at 4°C in the dark.
- **Standards:** Prepare the top (100 µg/ml) standard by diluting the Stock (5 mg/ml) Standard Solution in Assay Buffer in a ratio of 1 : 49 (e.g. to prepare 1 ml of top (100 µg/ml) standard, mix 20 µl Stock (5 mg/ml) Standard Solution with 980 µl Assay Buffer). The top (100 µg/ml) standard can only be stored for up to 3 days at 4°C in the dark, so prepare only as much standard as required for the planned assay.

  Prepare a 7-point standard curve as follows: Label 7 tubes with 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml. Add 20 µl, 40 µl, 60 µl, 80 µl, 120 µl, 160 µl, and 200 µl of top (100 µg/ml) standard to the 100 µg/ml, 80 µg/ml, 60 µg/ml, 40 µg/ml, 30 µg/ml, 20 µg/ml, and 10 µg/ml tubes respectively, followed by 180 µl, 160 µl, 140 µl, 120 µl, 80 µl, 40 µl, and 0 µl of Assay Buffer, to prepare Standard Dilutions with concentrations 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml. These volumes are summarized in the following table:

| **Standard Dilution (µg/ml)** | **100** | **80** | **60** | **40** | **30** | **20** | **10** |
|---|---|---|---|---|---|---|---|
| **100 µg/ml Standard (µl)** | 200 | 160 | 120 | 80 | 60 | 40 | 20 |
| **Assay Buffer (µl)** | 0 | 40 | 80 | 120 | 140 | 160 | 180 |

For the blank, or 0 µg/ml standard, use pure Assay Buffer. The volume of each standard will be 200 µl.

**Note:**

- Allow all reagents to equilibrate to room temperature before use.
- If there is any precipitate present in the Chromogenic Reagent Diluent, heat the vial to 37°C and swirl gently to dissolve the precipitate. Allow the vial to cool back to room temperature before use.
- If there are any crystals in the Saline Solution, heat the vial to up to 60°C and swirl gently to dissolve the crystals. Allow the vial to cool back to room temperature before use.
- Standard dilutions below the top (100 µg/ml) cannot be stored, and should be discarded after use. Prepare a fresh standard curve each time an assay is run.
- Particles suspended in the Matrix Solution may settle during storage. Before use, mix the Matrix Solution well to resuspend any sediment.

## B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Mark microcentrifuge tubes for each standard, sample, and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 80 µl of sample to each sample tube, and 80 µl of the same sample to its corresponding control tube.
3. Add 40 µl of Assay Buffer to all tubes.
4. Add 80 µl of Matrix Solution to all tubes.
5. Mix fully, then incubate all tubes at 37°C for 60 minutes in the dark.
6. Immerse the tubes in a boiling water bath for 5 minutes.
7. Centrifuge the tubes at 10,000 × g for 5 minutes at 25°C. Carefully collect the supernatant, and keep on ice.
8. Mark positions on the 96-well microplate for each standard, sample, and control.
9. Add 100 µl of each standard dilution to its corresponding standard tube.
10. Transfer 100 µl of each sample and control supernatant to fresh sample and control tubes.
11. Add 20 µl of Saline Solution to all tubes.
12. Mix the control tubes fully, then stand at 25°C for 5 minutes.
13. Mix the standard tubes fully, then immerse in a boiling water bath for 5 minutes. Allow the tubes to cool to room temperature.
14. Mix the sample tubes fully, then immerse in a boiling water bath for 5 minutes. Allow the tubes to cool to room temperature.
15. Add 300 µl of Working Chromogenic Reagent to all tubes, then incubate for 20 minutes at 37°C in the dark.
16. Immediately transfer 200 µl of the resulting solution in each tube to the corresponding wells on the 96-well microplate. *Avoid foaming or bubbles.*
17. Measure the OD of each well with a microplate reader at 585 nm.

**Note:**

- For best results, after the standards, samples, and controls have been incubated with the Working Chromogenic Reagent, add the resulting solutions to the microplate and measure the OD values as quickly as possible.

### C. Calculation of Results

Plot the standard curve, using the mean OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the activity of Chitinase in each sample well can be derived with the following formula:

#### 1. Tissue and cell lysate samples:

One unit of Chitinase activity is defined as the amount required for 1 mg of total tissue or cell protein to produce 1 µg of product per hour at 37°C:

$$\text{Chitinase activity (U/mg protein)} = \mathrm{F} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b}) \times \mathrm{V_{Reaction}}}{\mathrm{a} \times \mathrm{V_{Sample}} \times \mathrm{C_{Protein}} \times \mathrm{t}}$$

where:

| | |
|---|---|
| $\mathrm{OD_{Sample}}$ | OD value of sample |
| $\mathrm{OD_{Control}}$ | OD value of control |
| $\mathrm{V_{Reaction}}$ | Volume of reaction (200 µl) |
| $\mathrm{V_{Sample}}$ | Volume of sample added to the reaction (80 µl) |
| $\mathrm{C_{Protein}}$ | Concentration of protein in sample (mg/ml) |
| a | Gradient of the standard curve ($y = \boldsymbol{a}x + b$) |
| b | Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$) |
| t | Time of the enzymatic reaction (1 hour) |
| F | The dilution factor of sample |



## Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.