Beta-1,3-Glucanase Assay Kit

Catalog No.: abx097994

Size: 100 Assays

Storage: Store all kit components at 4°C.

Application: For quantitative detection of Beta-1,3-Glucanase activity in tissue homogenate and cell lysates.

Detection Range: 0.3 mmol/L – 3 mmol/L

Introduction: Beta-1,3-Glucanase is an enzyme which is found mainly in plants. It hydrolyzes beta-1,3-glucosidic bonds found in beta-1,3-glucans such as laminarin. It is upregulated in response to pathogens or stress.

Beta-1,3-Glucanase hydrolyzes beta-1,3-glucosidic bonds to generate a reducing sugar, which reacts with a dye reagent. The concentration of the reaction product is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 540 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Dye Reagent: 10 ml
4. Standard: 1 vial
5. Substrate: 1 vial
6. Plate Sealer: 3

Materials Required But Not Provided

1. Microplate reader (540 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Mortar
6. Centrifuge and centrifuge tubes
7. Timer
8. Ice
9. Sonicator

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 5 ml of distilled water into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

- **Standard Solution**
  Add 1 ml of distilled water to the Standard vial and mix thoroughly. Add 0.3 ml of this solution to 0.7 ml of distilled water to prepare a 1 ml Standard Solution with concentration 3 mmol/L.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 12,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

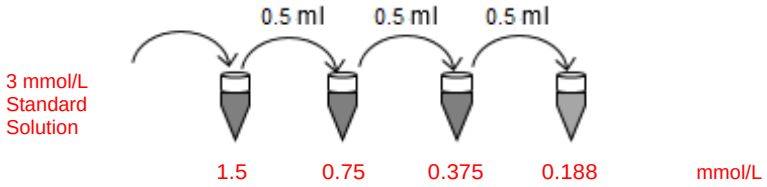
- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice. Centrifuge at 12,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 4 tubes with 1.5 mmol/L, 0.75 mmol/L, 0.375 mmol/L and 0.188 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 5 mmol/L standard solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard, control, and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 50 µl of sample to the sample wells.
4. Add 50 µl of distilled water to the control wells.
5. Add 50 µl of Substrate Solution to the sample wells and the control wells. At this stage, the standard and blank wells should be empty with no liquid.
6. Tap the plate gently to mix. Incubate at 37°C for 10 minutes.
7. Add 100 µl of prepared standard solutions to the standard wells.
8. Add 100 µl of distilled water to the blank wells.
9. Add 100 µl of Dye Reagent to all wells.
10. Tap the plate gently to mix. Incubate at 90°C for 10 minutes.
11. Read and record absorbance at 540 nm.

C. Calculations

One unit of Beta-1,3-Glucanase activity is defined as the amount of enzyme required to produce 1 µmol of reducing sugar per minute.

Beta-1,3-Glucanase activity per mg of protein:

$$\mathbf{Beta-1,3-Glucanase\ (U/mg)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times C_{Protein} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{0.6}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Beta-1,3-Glucanase activity per g of sample:

$$\mathbf{Beta-1,3-Glucanase\ (U/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{0.6}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (3 mmol/L = 3 µmol/ml)
$\mathbf{T}$	Reaction time (10 minutes)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{V_{Assay}}$	Volume of assay buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.05 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.1 ml)