Endo-Beta-Mannanase Assay Kit

Catalog No.: abx298907

Size: 100 Assays

Storage: Store the Positive Control at -20°C and all other kit components at 4°C.

Application: For quantitative detection of Endo-Beta-Mannanase activity in tissue homogenates, cell lysates and other biological fluids.

Detection Range: 1 mmol/L – 10 mmol/L

Introduction: Endo-Beta-Mannanase is an enzyme which catalyzes the hydrolysis of (1->4)-beta-D-mannosidic linkages in mannans, galactomannans and glucomannans. The cleavage occurs at random internal sites within the chain.

Endo-Beta-Mannanase reacts with 3,5-dinitrosalicylic acid to generate a red-brown reaction product. 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. Positive Control: 1 vial
7. Plate Sealer: 3

Materials Required But Not Provided

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

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 8 ml of Assay Buffer 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 to prepare the Standard Solution with concentration 10 mmol/L. Ensure that the Standard has completely dissolved prior to use.
- **Positive Control Solution**
  Add 0.1 ml of Assay Buffer to the Positive Control vial and mix thoroughly to prepare the Positive Control Solution. Ensure that the Positive Control has completely dissolved prior to use.

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 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

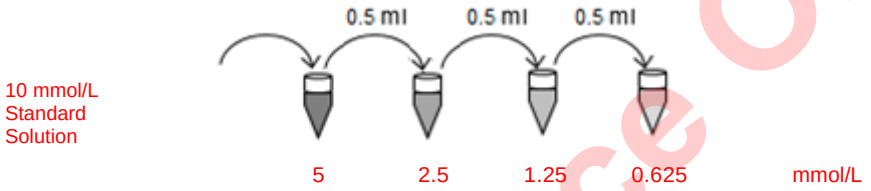
- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

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 5 mmol/L, 2.5 mmol/L, 1.25 mmol/L and 0.625 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 (2.5 mmol/L) 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, positive 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 20 µl of sample to the sample wells.
4. Add 20 µl of Assay Buffer to the control wells.
5. Add 20 µl of Positive Control Solution to the positive control wells.
6. Add 80 µl of Substrate Solution to the sample, control, and positive control wells. At this stage, the standard and blank wells should be empty with no liquid.
7. Tap the plate gently to mix. Incubate at 37°C for 10 minutes.
8. Add 100 µl of prepared standard solutions to the standard wells.
9. Add 100 µl of distilled water to the blank wells.
10. Add 100 µl of Dye Reagent to all wells.
11. Tap the plate gently to mix. Incubate at 90°C for 10 minutes.
12. Read and record absorbance at 540 nm.

C. Calculations

One unit of Endo-Beta-Mannanase activity is defined as the amount of enzyme required to produce 1 µmol of mannose per minute.

Endo-Beta-Mannanase activity per mg of protein:

$$\mathbf{Endo-Beta-Mannanase\ (U/mg)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times C_{Protein} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{5}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Endo-Beta-Mannanase activity per g of sample:

$$\mathbf{Endo-Beta-Mannanase\ (U/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{5}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Endo-Beta-Mannanase activity per 10^4 cells or bacteria:

$$\mathbf{Endo-Beta-Mannanase\ (U/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times N \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{5}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (10 mmol/L = 10 µmol/ml)
T	Reaction time (10 minutes)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of assay buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.02 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.1 ml)