Cellulase Assay Kit

Catalog No.: abx298861

Size: 100 Assays

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

Application: For quantitative detection of Cellulase activity in tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids.

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

Introduction: Cellulase is a blanket term for enzymes that can digest cellulose; β1-4 glucose polymers of glucose which act as one of the main structural materials in plant cell walls. Plants have very few cellulases, and it is thought they use these to remodel cellulose structures during growth. Fungi and bacteria have considerably more cellulases to help them digest plant matter. Some invertebrates, such as snails and termites, have cellulases; vertebrates like sheep and horses do not, and must rely on cellulases produced by symbiotic bacteria. Cellulases are used industrially in the production of biofuels, coffee, and textiles.

Abbexa's Cellulase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating cellulase activity. The enzyme catalyzed reaction product has an absorption maximum at 540 nm. The intensity of the color is proportional to the activity of the cellulase enzyme(s), which can then be calculated.

Kit components

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

Materials Required But Not Provided

1. Microplate reader (540 nm)
2. 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

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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. Ensure that the Standard has completely dissolved. 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.
- **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 10,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

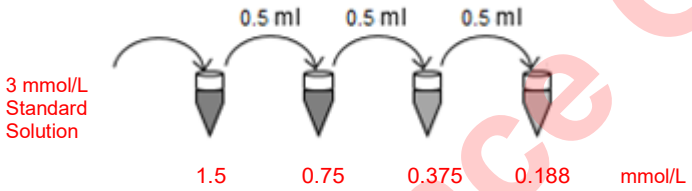
- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice, then allow to stand for 2 hours. Centrifuge at 10,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

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 3 mmol/L standard solution to the 1st tube (1.5 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, blank, control, and positive control wells and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 10 µl of sample to the sample wells.
4. Add 10 µl of Assay Buffer to the control wells.
5. Add 10 µl of Positive Control Solution to the positive control wells.
6. Add 50 µl of Reaction Buffer to the sample wells, control wells, and positive control wells.
7. Add 40 µl of Substrate Solution to the sample wells, control wells, and positive control wells.
8. At this point, the standard wells and blank wells should be empty with no liquid. Tap the plate gently to mix. Incubate in a convection oven at 37°C for 10 minutes.
9. Add 100 µl of prepared standards to the standard wells.
10. Add 100 µl of distilled water to the blank wells.
11. Add 100 µl of Dye Reagent to all wells.
12. Tap the plate gently to mix. Incubate in a convection oven at 90°C for 10 minutes.
13. Read and record the absorbance at 540 nm.

C. Calculations

One unit of Cellulase activity is defined as the amount of enzyme required to produce 1 µmol of reducing sugar per minute.

Cellulase activity per mg of protein:

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

Cellulase activity per g of sample:

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

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Cellulase activity per 10^4 cells or bacteria:

$$\textbf{Cellulase}\ (\textbf{U}/\textbf{10}^{\textbf{4}}\ \textbf{cells}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{N} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{3}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (3 mmol/L = 3 µmol/ml)
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 (0.01 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.1 ml)

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