Beta-Glucosidase Assay Kit

Catalog No.: abx298863

Size: 96 tests

Detection Range: 0.06 U/L – 6.79 U/L

Sensitivity: 0.06 U/L

Storage: Store all components in the dark at -20°C.

Application: For detection and quantification of Beta-Glucosidase activity in fungal samples and tissue homogenates.

Introduction

Beta-Glucosidase is an enzyme which catalyses the breakdown of terminal, non-reducing beta-D-glucoside bonds in various glycosides and oligosaccharides to release beta-D-glucose.

Abbexa's Beta-Glucosidase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Beta-Glucosidase activity. Beta-Glucosidase catalyses the reaction of the substrate to produce chromogenic substances which have an absorbance maximum of 400 nm. The Beta-Glucosidase activity can be calculated using the change in absorbance over time.

Kit components

1. 96-well microplate
2. Substrate Solution: 5 ml
3. Buffer Solution: 50 ml
4. Extraction Solution: 2 × 50 ml
5. Standard (10 mmol/L): 2 ml
6. Plate sealer: 2

Materials required but not provided

1. Microplate reader (400 nm)
2. PBS (0.01 M, pH 7.4)
3. Ice
4. Mechanical homogenizer
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

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

- **Tissue Homogenates and Fungal Samples:** Carefully weigh 20 mg of sample and wash with cold PBS (0.01 M, pH 7.4). Add in 180 µl of Exctraction Solution and homogenize manually, using a mechanical homogenizer, at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C, then carefully remove the supernatant. Keep on ice and assay immediately.

  Note: To calculate Beta-Glucosidase activity in tissue homogenates and fungal samples using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (**abx097193, abx097194**).

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10 % Apple seed homogenates	50 – 60
10 % Spinach tissue homogenates	1
10 % Shiitake mushroom tissue homogenates	3 – 5
10 % Grapefruit seed homogenates	1
10 % Corn tissue homogenates	1
10 % Mushroom tissue homogenates	1
10 % Cabbage tissue homogenates	1
10 % Mouse small intestine tissue homogenates	1
10 % Pear seed homogenates	2 – 4

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 fungal samples and tissue homogenates.

2. Reagents

- **Substrate Working Solution:** Prepare enough Substrate Working Solution required for the amount of wells used. To prepare enough for 1 well, add 36 µl of Substrate Solution and 144 µl of Buffer Solution and mix thoroughly. Keep Substrate Working Solution in the dark and use within 24 hours.
- **Standard Solution (200 µmol/L):** Perpare enough Standard Solution (200 µmol/L) required for the amount of wells used. One set of standard dilutions requires 2000 µl of Standard Solution (200 µmol/L). Dilute the Standard Solution (10 mmol/L) 50-fold with Buffer Solution. For example, add 40 µl of Standard Solution (10 mmol/L) to 1960 µl Buffer solution to prepare 2000 µl of Standard Solution (200 µmol/L).
- **Standards:** Label 7 tubes with 200 µmol/L, 160 µmol/L, 140 µmol/L, 120 µmol/L, 80 µmol/L, 60 µmol/L, and 40 µmol/L. Add 500 µl, 400 µl, 350 µl, 300 µl, 200 µl, 150 µl, and 100 µl of Standard Solution (200 µmol/L) to the 200 µmol/L, 160 µmol/L, 140 µmol/L, 120 µmol/L, 80 µmol/L, 60 µmol/L, and 40 µmol/L tubes respectively, followed by 0 µl, 100 µl, 150 µl, 200 µl, 300 µl, 350 µl, and 400 µl of Buffer Solution, to prepare Standard Dilutions with concentrations 200 µmol/L, 160 µmol/L, 140 µmol/L, 120 µmol/L, 80 µmol/L, 60 µmol/L, and 40 µmol/L. These volumes are summarized in the following table:

Standard Dilution (µmol/L)	**200**	**160**	**140**	**120**	**80**	**60**	**40**
200 µmol/L Standard (µl)	500	400	350	300	200	150	100
Buffer Solution (µl)	0	100	150	200	300	350	400

For the blank, or 0 µmol/L standard, use pure Buffer Solution. The volume of each standard will be 500 µl.

B. Assay Procedure

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

1. Assign and record the positions of test wells for each standard and sample.
2. Add 20 µl of sample to each sample well.
3. Add 200 µl of each prepared standard dilution to the corresponding standard wells.
4. Add 180 µl of Substrate Working Solution to each sample well.
5. Tap the plate gently to mix for 5 seconds then measure the OD value of the sample wells with a microplate reader at 400 nm. Record this as A_1 (For example, $A_{1\ Sample}$).
6. Incubate at 37°C for 30 minutes then measure the OD values of each well with a microplate reader at 400 nm. Record these as A_2 (For example, $A_{2\ Sample}$ and $A_{2\ Standard}$)

C. Calculation of Results

Plot the standard curve, using the OD ($A_{2\ Standard}$) values 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 Beta-Glucosidase in each sample well can be derived with the following formula:

1. Tissue Homogenates and Fungal Samples:

One unit of Beta-Glucosidase activity is defined as the amount required for 1 g of sample protein to produce 1 μmol of product per minute at 37°C.

$$\text{Beta-Glucosidase activity (U/g protein)} = \mathbf{F} \times \mathbf{10} \times \frac{(A_2 - A_1 - \mathrm{b})}{\mathrm{a} \times \mathrm{t} \times \mathrm{C_{Protein}}}$$

where:

$A_{2\ Sample}$	OD value of sample wells after reaction.
$A_{1\ Sample}$	OD value of sample wells before reaction.
$A_{2\ Standard}$	OD value of standard wells after reaction.
$C_{Protein}$	Concentration of protein in sample (g protein/L).
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 (30 minutes).
F	The dilution factor of sample before assay.
10	The dilution factor of protein concentration when added to the reaction system.

Technical Support

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