Beta-Galactosidase (GLB1) Assay Kit

Catalog No.: abx298865

Size: 96 tests

Storage: Store the powdered Substrate at -20°C. Store all other kit components at 4°C.

Detection Range: 16.2 U/L – 1200 U/L

Application: For quantitative detection of Beta-Galactosidase (GLB1) activity in serum, plasma, tissue homogenates, cell culture supernatants, cell lysates, and other biological fluids.

Introduction: Beta-Galactosidase (GLB1) is a widespread glycoside hydrolase found in both plants and animals. It catalyzes the hydrolysis of the beta-glycosidic bond between galactose and a variety of organic moieties. In plants, galactose is the principal monomer in the hemicellulose strands that partly form plant cell walls, meaning GLB1 is heavily involved in plant metabolism and growth. In animals, GLB1 is used to break down various sugars for use in respiration. In addition, GLB1's inducible expression in *E. coli* through the *lacZ* gene, and its ability to activate the chromogen X-gal, has made Beta-Galactosidase a useful reporter enzyme in bioengineering applications.

Beta-Galactosidase catalyzes the hydrolysis of nitrophenyl-β-galactopyranoside, producing nitrophenol. The concentration of this reaction product is directly proportional to the enzyme activity, which can be determined by measuring the absorbance at 400 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 25 ml
3. Substrate: 1 vial
4. Reaction Buffer: 1.5 ml
5. Dye Reagent: 25 ml
6. Standard (20 mmol/L): 1 ml
7. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (400 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Hot water bath
5. Distilled water
6. Centrifuge and centrifuge tubes
7. Timer
8. Ice

Protocol

A. Preparation of Sample and Reagents

1. Sample

- **Serum and plasma:** Serum and plasma can be sampled directly. For samples with significant amounts of precipitate, centrifuge at 10,000 x g and then take the supernatant for analysis.
- **Tissue homogenates**: Carefully weigh a section of tissue, and place into a homogenizer tube – generally, 0.1 g of sample is sufficient. Aliquot Assay Buffer in a ratio of 1:9 (i.e., for 0.1 g of tissue, add 0.9 ml of Assay Buffer). Homogenize the tissue by hand on ice. Centrifuge at 10,000 x g for 10 minutes, then take the supernatant for analysis and keep on ice. If any precipitate is observed in the supernatant, centrifuge again until clear.
- **Cell and Bacterial samples**: Collect the cells into a centrifuge tube, and wash with 0.01 M PBS twice. Centrifuge at 1000 x g for 10 minutes to precipitate the cells, then remove and discard the supernatant. Add 200 µl of Assay Buffer for every 10^6 (1,000,000) cells. Sonicate on ice 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 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

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 using the Assay Buffer, then carry out the assay procedure. The concentrations of each sample must fall within the kit's stated range (16.2 – 1200 U/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Mouse liver tissue homogenate	1
10% Rat brain and heart tissue homogenate	1
10% Rat liver tissue homogenate	1 – 3
10% Rat kidney tissue homogenate	3 – 5
Human serum	1

2. Reagents

- **Substrate Solution**: Dissolve the powdered Substrate with 2 ml of Assay Buffer in a 90 – 100°C water bath, and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved, and restore to room temperature before use. The prepared Substrate Solution can be stored up to 1 week in the dark at 4°C.
- **1 mmol/L Standard Solution:** Dilute the concentrated 20 mmol/L Standard with Distilled water in a ratio of 1:19 (i.e. to the 1 ml of 20 mmol/L Standard, add 19 ml of Distilled water).
  The diluted Standard Solution can be stored up to 1 week in the dark at 4°C.
- **Standard Dilutions:** Label 7 tubes with 0.8 mmol/L, 0.7 mmol/L, 0.6 mmol/L, 0.5 mmol/L, 0.4 mmol/L, 0.2 mmol/L, and 0.1 mmol/L. Add 160 µl, 140 µl, 120 µl, 100 µl, 80 µl, 40 µl, and 20 µl of Standard Solution (1 mmol/L) to the 0.8 mmol/L, 0.7 mmol/L, 0.6 mmol/L, 0.5 mmol/L, 0.4 mmol/L, 0.2 mmol/L, and 0.1 mmol/L tubes respectively, followed by 40 µl, 60 µl, 80 µl, 100 µl, 120 µl, 160 µl, and 180 µl of Distilled water, to prepare the Standard Dilutions with concentrations 0.8 mmol/L, 0.7 mmol/L, 0.6 mmol/L, 0.5 mmol/L, 0.4 mmol/L, 0.2 mmol/L, and 0.1 mmol/L ml. These volumes are summarized in the following table:

Standard Dilution (mmol/L)	0.8	0.7	0.6	0.5	0.4	0.2	0.1
1 mmol/ml Standard (µl)	160	140	120	100	80	40	20
Distilled water (µl)	40	60	80	100	120	160	180

For the blank, or 0 µmol/ml standard, use pure Distilled water. The volume of each standard will be 200 µl.

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. Set positions for the sample, control, and standard wells on the 96 well microplate. Each sample tested will require a corresponding control well. *We recommend setting up each standard and sample in duplicate*.
2. Add 20 µl of sample to the sample and control wells.
3. Add 20 µl of diluted Standard Solution to the standard wells.
4. Add 30 µl of Substrate Solution to the sample wells.
5. Add 30 µl of Assay Buffer to the control and standard wells.
6. Add 10 µl of Reaction Buffer to each well. All the wells should now contain 60 µl of solution.
7. Mix fully with a microplate shaker, and then incubate at 37°C for 40 minutes.
8. Add 140 µl of Dye Reagent to each well.
9. Mix fully with a microplate shaker, and then incubate in the dark at 37°C for 10 minutes.
10. Read and record absorbance at 400 nm.

C. Calculations

Plot the standard curve, using the OD of the standard dilutions on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = mx + b$. Based on this curve, the amount of Beta-Galactosidase (GLB1) in each sample well can be derived according to sample type with the following formulae:

Beta-Galactosidase activity per gram of tissue or cell protein:

For this calculation, the concentration of total protein in the samples must be known. One unit of Beta-Galactosidase is defined as the amount of enzyme in 1 g of total sample protein that can produce 1 µmol of p-nitrophenol per hour at 37°C.

$$\mathbf{GLB1\ (U/g_{PROT.})} = \mathbf{F} \times \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}) - \mathbf{b}}{\mathbf{m} \times \mathbf{C_{Pr}} \times \mathbf{T}} \times \mathbf{1000}$$

Beta-Galactosidase activity per gram of tissue sample:

One unit of Beta-Galactosidase is defined as the amount of enzyme in 1 kg of tissue that can produce 1 µmol of p-nitrophenol per hour at 37°C.

$$\mathbf{GLB1\ (U/kg)} = \mathbf{F} \times \mathbf{V} \times \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}) - \mathbf{b}}{\mathbf{m} \times \mathbf{W} \times \mathbf{T}} \times \mathbf{1000}$$

Beta-Galactosidase activity per liter of serum or plasma:

One unit of Beta-Galactosidase is defined as the amount of enzyme in 1 L of serum or plasma that can produce 1 µmol of p-nitrophenol per hour at 37°C.

$$\mathbf{GLB1\ (U/L)} = \mathbf{F} \times \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}) - \mathbf{b}}{\mathbf{m} \times \mathbf{T}} \times \mathbf{1000}$$

where:

$\mathbf{OD_{Sample}}$	Absorbance of the sample well at 400 nm
$\mathbf{OD_{Control}}$	Absorbance of the corresponding control well at 400 nm
$\mathbf{F}$	Dilution factor of the sample
$\mathbf{C_{Pr}}$	Concentration of protein (in g/L)
$\mathbf{T}$	Reaction time (0.5 hours)
$\mathbf{W}$	Weight of the sample (in g; recommended: 0.1 g)
$\mathbf{V}$	Volume of Assay Buffer used during homogenization (in ml; recommended: 0.9 ml)
$\mathbf{m}$	The gradient of the standard curve ($\boldsymbol{y = mx + b}$)
$\mathbf{b}$	The intercept of the standard curve ($\boldsymbol{y = mx + b}$)