Beta-Glucuronidase Assay Kit

Catalog No.: abx298967

Size: 100 Assays

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

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

Detection Range: 5 µmol/L – 500 µmol/L

Introduction: Beta-Glucuronidase is a hydrolytic enzyme that breaks down carbohydrates by cleaving the terminal beta-D-glucuronic acid residue from the non-reducing terminus of a mucopolysaccharide chain. In humans, these enzymes are found in the lysosome of many tissues. Loss of beta-Glucuronidase activity results in a metabolic disease known as Sly syndrome. As the expression and activity of beta-Glucuronidase can vary substantially between tissue types and disease states, these enzymes have been used to achieve targeted activation of glucuronidated prodrugs. Knowledge of beta-Glucuronidase activity can be used to determine whether the prodrug or active form will predominate.

Abbexa's Beta-Glucuronidase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Beta-Glucuronidase activity. The reaction product has an absorption maximum at 560 nm. The intensity of the color is proportional to the activity of Beta-Glucuronidase, which can then be calculated.

Kit components

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

Materials Required But Not Provided

1. Microplate reader (560 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Mortar
8. Incubator
9. Ethanol

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 2 ml of Reaction Buffer into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use. Unused Substrate Solution can be aliquoted and stored at -20°C for up to 1 month after reconstitution.

- **Standard Solution**
  Add 1 ml of ethanol into the Standard vial and mix thoroughly. Ensure that the Standard has completely dissolved. Add 100 µl of this solution into 900 µl of ethanol to prepare the Standard Solution (concentration 500 µmol/L). Unused Standard Solution can be aliquoted and stored at -20°C for up to 1 month after reconstitution.

- **Positive Control Solution**
  Add 1 ml of Assay Buffer into the Positive Control vial and mix thoroughly to prepare the Positive Control Solution. Ensure that the Positive Control has completely dissolved prior to use. Unused Positive Control Solution can be aliquoted and stored at -80°C for up to 1 month after reconstitution.

2. Sample

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

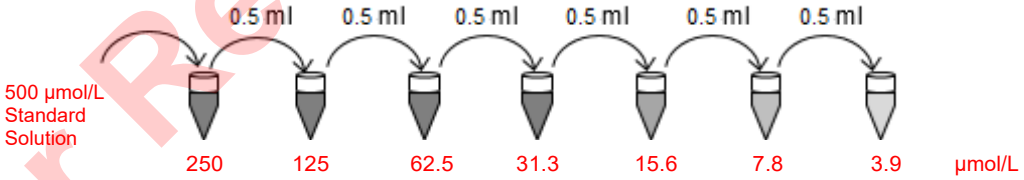
- **Liquid samples**
  Liquid samples such as serum, plasma, and other biological fluids, can be used directly.

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 7 tubes with 250 µmol/L, 125 µmol/L, 62.5 µmol/L, 31.3 µmol/L, 15.6 µmol/L, 7.8 µmol/L, and 3.9 µmol/L. Aliquot 0.5 ml of ethanol into each tube. Add 0.5 ml of 500 µmol/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, blank, control, and positive control wells on the 96 well microplate 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 Positive Control Solution to the positive control wells.
5. Add 10 µl of prepared standards to the standard wells.
6. Add 10 µl of distilled water to the control wells.
7. Add 20 µl of distilled water to the standard wells.
8. Add 30 µl of distilled water to the blank wells.
9. Add 70 µl of Reaction Buffer to all wells.
10. Add 20 µl of Substrate to the sample, control, and positive control wells. At this stage, all wells used should have a volume of 100 µl each.
11. Gently tap the plate to mix or use a microplate shaker. Cover the plate with a plate sealer and incubate at 37°C for 3 hours.
12. Add 100 µl of Dye Reagent to all wells.
13. Gently tap the plate to mix or use a microplate shaker. Read and record absorbance at 560 nm.

C. Calculations

One unit of Beta-Glucuronidase activity is defined as the amount of enzyme required to produce 1 nmol of phenolphthalein acid per hour.

Beta-Glucuronidase activity per mg of protein:

$$\textbf{Beta} - \textbf{Glucuronidase}\ (\textbf{U/mg}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{C_{Protein}} \times \mathbf{V_{Sample}} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{166.67}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Beta-Glucuronidase activity per g of sample:

$$\textbf{Beta} - \textbf{Glucuronidase}\ (\textbf{U/g}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}} \times \mathbf{V_{Assay}}}{\mathbf{W} \times \mathbf{V_{Sample}} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{166.67}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Beta-Glucuronidase activity per ml of serum or plasma:

$$\textbf{Beta} - \textbf{Glucuronidase}\ (\textbf{U/ml}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \mathbf{166.67} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (500 µmol/L= 500 nmol/ml)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{V_{Assay}}$	Volume of Assay Buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.01 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.01 ml)
$\mathbf{T}$	Reaction Time (3 hours)