beta-Glucuronidase Assay Kit (BA0217)

Product Datasheet | SKU BA0217

Description

Beta-glucuronidase is an enzyme that catalyses the hydrolysis of beta-glucuronide bonds. In humans it hydrolyses beta-D-glucuronic acids from glycoproteins and mucopolysaccharides such as heparan sulfate, and its activity is important for drug metabolism because drugs are often conjugated to glucuronic acid for more water-soluble delivery. Bilirubin clearance is also facilitated by glucuronidation, and the enzyme is frequently used as a reporter to track gene expression. This kit uses a beta-D-glucuronide substrate that fluoresces upon hydrolysis and is read at 365/450 nm, with fluorescence proportional to enzyme activity.

Specifications

Detection Method	Fluorimetric, kinetic; read at lambda ex/em = 365/450 nm
Detection Range	1x10-4 U/L to 8 U/L beta-glucuronidase activity
Sample Type	Tissue lysate, cell lysate, serum, bacterial samples
Species Reactivity	All
Assay Time	Approximately 45 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Store all kit components at -20 degrees C.
Shelf Life	6 months after receipt
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Substrate	4 mL	-20 degrees C
Standard	100 uL	-20 degrees C
Stop Reagent	1.5 mL	-20 degrees C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0217	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. This assay is based on a kinetic reaction, so addition of substrate to samples should be quick and mixing brief but thorough; use of a multi-channel pipettor is recommended and assays can be run at room temperature or 37 degrees C.
2. For sample preparation, avoid surfactants such as SDS or Triton 100-X.
3. For tissue, rinse in phosphate buffered saline (pH 7.4) to remove blood prior to dissection, then homogenise 50 mg tissue in approximately 200 microlitres of lysis buffer.
4. For cell lysate, collect cells by centrifugation at 2,000 x g for 5 minutes at 4 degrees C, use a rubber policeman rather than proteolytic enzymes for adherent cells, then homogenise or sonicate in cold lysis buffer; samples may be stored at -20 to -80 degrees C for at least one month.
5. Equilibrate all kit reagents to room temperature and invert or gently vortex to ensure they are mixed.
6. Prepare a 100 uM Premix by mixing 5 microlitres of the provided Standard with 995 microlitres of distilled water, then prepare standards as shown in the standard preparation table.

7. For tissue lysates dilute samples 5-10 fold with deionised water prior to assay, and for serum samples a 15-fold dilution may be required.
8. Transfer 10 microlitres of standards and 10 microlitres of samples into wells of a black flat-bottom 96-well plate.
9. Add 40 microlitres of substrate to each well, tap the plate to mix and incubate at room temperature or 37 degrees C for 30 minutes.
10. Add 15 microlitres of stop reagent to each well, tap the plate to mix and incubate for 15 minutes.
11. Read fluorescence at lambda ex/em = 365/450 nm.
12. This assay procedure can be readily miniaturised for 384- or 1536-well plates.

Standard Preparation

No	Premix + H2O Vol (uL)	Standard (uM)
1	100 uL + 0 uL (100)	100
2	60 uL + 40 uL (100)	60
3	30 uL + 70 uL (100)	30
4	0 uL + 100 uL (100)	0

Calculation

BG Activity = [(F_SAMPLE - F_BLANK) / (Time x Slope)] x n (U/L), where F_SAMPLE is the RFU value for each sample and F_BLANK is the RFU value of the water (standard #4). Slope is the slope of the standard curve and Time is the incubation time (30 min). n is the sample dilution factor. Unit definition: 1 Unit (U) of beta-glucuronidase will catalyse the conversion of 1 micromole of the fluorescent glucuronide at 37 degrees C and pH 5.0. Note: if a sample exceeds 8 U/L calculated activity, use a shorter incubation time or dilute samples in water and repeat; for samples below 0.2 U/L, the incubation time can be extended up to 2 hours for greater sensitivity.

Notes

- This assay is based on a kinetic reaction; to ensure identical incubation times, addition of substrate should be quick and mixing brief but thorough.
- Avoid surfactants such as SDS or Triton 100-X in sample preparation.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.