QuantiChrom™ β-Glucuronidase Assay Kit (DGCD-100)

Quantitative Fluorimetric Kinetic β-Glucuronidase Activity Determination

DESCRIPTION

BETA-GLUCURONIDASE (BG) is an enzyme which catalyzes the hydrolysis of β-glucuronide bonds. In humans, β-glucuronidase catalyzes the hydrolysis of β-D-glucuronic acids from glycoproteins and mucopolysaccharides such as heparan sulfate. β-glucuronidase activity is important for drug metabolism as drugs are often conjugated to glucuronic acid to make for a more water soluble delivery system, relying on subsequent β-glucuronidase activity to release the active drug. Bilirubin clearance is also facilitated by glucuronidation. GUSB is often used as a reporter gene to track gene expression through β-glucuronidase activity. BioAssay Systems' fluorimetric BG assay uses a β-D-glucuronide substrate which fluoresces upon hydrolysis with β-glucuronidase and is read at 365/450 nm.

KEY FEATURES

Highly Sensitive. The fluorimetric nature of the kit detects $1x10^{-4}$ U/L to 8 U/L BG activity in 96 well plate with only 10 µL sample within 30 min.

Versatile. This assay has been validated to work with both animal and *E. coli* derived β-glucuronidase.

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of beta glucuronidase enzyme activity in tissue and bacterial samples.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Substrate: 4 mL **Standard:** 100 µL
Stop Reagent: 1.5 mL

Storage conditions. The kit is shipped on ice. Store all kit components at -20 °C. Shelf life of 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of substrate to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be run at room temperature or 37°C.

Sample Preparation: we recommend to avoid surfactants such as SDS or Triton 100-X in sample preparation.

Tissue: Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL lysis buffer.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold lysis buffer. All samples can be stored at –20 to –80°C for at least one month.

Reagent Preparation: Equilibrate all kit reagents to room temperature. Invert or gently vortex tubes and bottles to ensure reagents are mixed.

Standard Preparation: Prepare 100 µM Premix by mixing 5 µL of the provided Standard with 995 µL dH_2O. Prepare the standards as shown in the following table.

No	Premix + H_2O	Vol (µL)	Standard (µM)
1	100µL + 0µL	100	100
2	60µL + 40µL	100	60
3	30µL + 70µL	100	30
4	0µL + 100µL	100	0

Sample Preparation: For tissue lysates we recommend you dilute samples 5-10 fold with deionized water prior to assay. For serum samples a 15-fold dilution may be required.

1. Transfer 10 µL Standards and 10 µL samples into wells of a black flat bottom 96 well plate.
2. Add 40 µL substrate to each well. Tap plate to mix. Incubate plate at RT or 37°C for 30 minutes.
3. Add 15 µL stop reagent to each well. Tap plate to mix. Incubate for 15 minutes.
4. Read Fluorescence at λex/em = 365/450nm.

This assay procedure can be readily miniaturized for 384- or 1536-wells.

CALCULATION

BG activity can then be calculated as follows:

$$\text{BG Activity} = \frac{F_{SAMPLE} - F_{BLANK}}{\text{Time} \cdot \text{Slope}} \times n \ \ (\text{U/L})$$

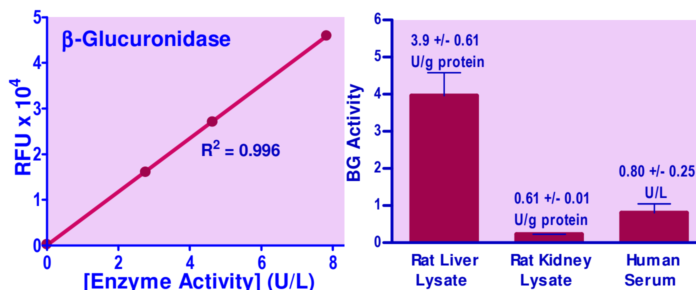
where F_{SAMPLE} is the RFU value for each sample and F_{BLANK} is the RFU value of the water (standard #4). Slope is the slope of the standard curve and Time is the incubation time (30 min). *n* is the sample dilution factor.

Unit definition: 1 Unit (U) of BG will catalyze the conversion of 1 µmole of the fluorescent glucuronide at 37°C and pH 5.0.

Note: If sample exceeds 8 U/L calculated BG activity, either use a shorter incubation time or dilute samples in water and repeat the assay. For samples with calculated BG activity < 0.2 U/L, the incubation time can be extended up to 2 hours for greater sensitivity.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), black flat-bottom 96-well plates (e.g. VWR cat# 89089-582), centrifuge tubes and plate reader.



LITERATURE

1. Matsushita-Oikawa, H, et al. (2006) Novel carbohydrate-binding activity of bovine liver β-glucuronidase toward lactose/N-acetyllactosamine sequences. Glycobiology, *16*(10), 891-901.
2. Marathe, S. V., & McEwen, J. E. (1995). Vectors with the gus reporter gene for identifying and quantitating promoter regions in Saccharomyces cerevisiae. Gene, *154*(1), 105-107.