Tribioscience

Beta-Glucuronidase Activity Colorimetric Assay (TBS2110, Store at -20℃)

Catalog Number	Kit Size
TBS2110-100	100 assays
TBS2110-200	200 assays

Description

β-Glucuronidases is a kind of lysosomal enzyme which belongs to the glycosidase family. It plays a critical role during the process of metabolic and detoxification, especially for the breakdown of complex carbohydrates in the human body. β -Glucuronidase level imbalance is related to many diseases, such as cancer, diabetes, liver and detoxification disorders, inflammation, autoimmune, and neuron degenerative diseases. Therefore, it is important to detect activity of β-Glucuronidase in samples to elucidate the pathology of diseases.

Tribioscience's β-Glucuronidase Activity Colorimetric Assay provides a simple and sensitive method for monitoring glucuronidase activity in biological samples (tissue, cells, serum, urine). This assay uses a synthetic p- nitrophenol derivative (R-pNP) as its substrate. The substrate releases a chromophore pNP under β-Glucuronidase, which can be measured at absorbance (OD 405 nm). The assay can detect as low as 50 µU of glucuronidase activity in a variety of samples. The reaction was shown in **Fig. 1** as below.

Synonyms: β-Glucuronidase; β-D Glucuronide glucuronosohydrolase; GUSB; Ac2-223; beta-G1; BG; glucuronidase beta; Gus.

Fig.1

Substrate + H_2O →(β-Glucuronidase)→ ☆ + ● 405 nm

Applications

Determination of β-Glucuronidases activity in biological samples.

Key Features

Fast and sensitive: Linear detection range (20 µL sample): 0.05 to 50 U/L for a 30-minute reaction at 37˚C.

High throughput: Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Kit Contents

Component	100x RXNS	200x RXNS
BG Substrate	9 mL	18 mL
pNP Standard (10mM)	0.1 mL	0.2 mL
BG Positive control (10x)	10 µL	20 µL
BG Stop Reagent	11 ml	22mL
BG Assay Buffer	10 ml	20 mL

Storage Conditions

The kit is shipped on ice and should be stored at -20°C for long-term storage. Shelf life of 24 months after receipt.

Procedures

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate and Stop Reagent to samples should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Sample Preparation: Serum and plasma can be assayed directly. For urine samples containing precipitation, centrifuge at 10,000 x g, 4°C for 3 minutes and assay the supernatant.
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 PBS, approximately one million cells per mL. Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

Reagent Preparation: Equilibrate all components to room temperature. Briefly vortex gently (except positive control) or pipette up and down all components to ensure fresh reconstitution.

Reaction Preparation:

1. Label tubes as #1 through #8 as below diagram.
2. Add 120 µL of Assay Buffer to Std1, and 75 µL to Std2 to 8.
3. Pipet 30 µL of 10 mM standard stock into Std#1. Then make 2x series dilution in Std2 through 7 with addition of 75 µL. Std8 is 1x Assay Buffer alone as a standard 0. The standard concentration in tube 1 through 7 will be 2000,1000, 500, 250, 125, 62.5 and 31.25µM, Tube#8 is Standard 0 as blank.

Table 1.

	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	120	75	75	75	75	75	75	75
Addition	**Stock**	**Std1**	**Std2**	**Std3**	**Std4**	**Std5**	**Std6**	
Addition Vol. (µL)	30	75	75	75	75	75	75	0
Final Conc (µM)	2000	1000	500	250	125	62.5	31.25	0

4. Positive Control: Add 90 µL of assay buffer to 10x positive control Stock and gently mix as positive control
5. Transfer 20 µL of each sample, blank, positive control, and standards into two separate wells.
6. Add 80 µL of the substrate solution to all sample, positive control, and blank wells. Add 80 µL of Assay Buffer to each

standard well *(Note: Do not add substrate in the standard)*. Tap plate briefly to mix.

7. Incubate at 37°C for 30-60 minutes.
8. Add 100 µL of Stop Reagent to all wells. Tap plate briefly to mix.
9. Read OD at 405nm in endpoint model.

Calculation

Subtract blank OD (Standard 0, #8) from the standard OD values and plot the ΔOD against standard concentrations. Determine the slope, calculate β-glucuronidase activity using the equation below:
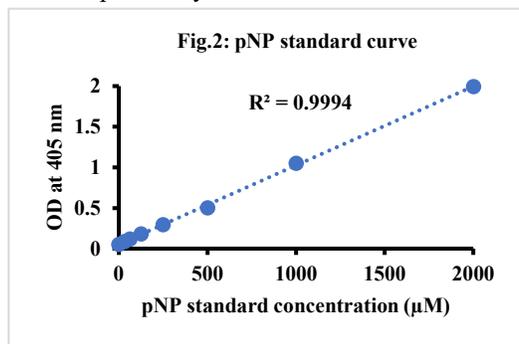
B-Glucuronidase Activity (U/L):

$$DF * (OD_{SAMPLE} - OD_{BLANK})/ (t * Slope)$$

where OD_{SAMPLE} is the OD405nm value for each sample and OD_{BLANK} is the OD405nm value of the sample blank. Slope is the slope of the linear regression fit of the standard points and t is the reaction time (30 min). DF is the dilution factor.

Unit definition: One Unit (U) β-glucuronidase catalyzes the conversion of 1 µmole of 4-nitrophenyl -β-D-glucuronide to 4-nitrophenol and β-glucuronic acid per min at 37°C

Typical Data

This standard curve is provided for demonstration only as a reference in **Fig.2.** A standard curve should be generated for each set of samples assayed.

Fig.2: pNP standard curve

$R^2 = 0.9994$

OD at 405 nm vs pNP standard concentration (µM)

Beta-Glucuronidase activity is validated with the kit. The data is shown in **Fig.3.**

Fig. 3: Glucuronidase positive control curve

$R^2 = 0.9998$

OD at 405 nm vs Glucuronidase units/well
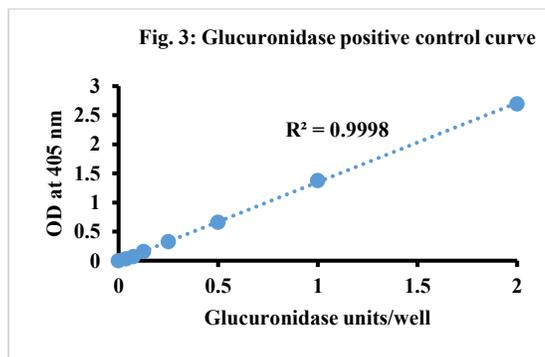
Relative Products

Tryptase activity colorimetric assay (TBS2101)
Hex activity colorimetric assay (TBS2105)
Caspase-3 Fluorometric Assay kit (TBS3230)
Cytochrome C Oxidase Activity Assay (TBS2115)
Fast Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
Glucose Oxidase Activity Colorimetric/Fluorometric Assay (TBS2088)
Non-esterified Fatty Acid Assy (TBS2203)
Glycerol Colorimetric / Fluorometric Assy (TBS2204)
Protein Assay Kits (TBS2005)
Cell Nuclear Extract kit (TBS6025)

Research use only.