Beta-Glucuronidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1167

(Version 1.4B)

Detection and Quantification of Beta-Glucuronidase(GGT) ActivityinUrine,Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Beta-Glucuronidase is hydrolytic enzyme responsible for the breakdown of carbohydrates. Specifically, beta-Glucuronidase cleave 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 tissue types. Loss of Beta-Glucuronidase activity results in a metabolic disease known as Sly syndrome. One pharmaceutical application for these enzymes is the metabolism of glucuronidated prodrugs into active pharmacological compounds. As expression and activities of beta-Glucuronidase vary substantially between tissue types and disease states, these enzymes have been used to achieve targeted activation of oncotherapeutic compounds, some of which may be toxic to healthy cells not associated with malignancy or disease. It is thus important to have knowledge of the beta-Glucuronidase activity in the tested sample to determine whether the prodrug or active form will predominate.

Beta-GlucuronidaseActivity ColorimetricMicroplate Assay Kitis designed to measure beta-glucuronidase activity in various samples in 96-well microplate. The color intensity at 560nm is directly proportional to beta-glucuronidaseactivity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	10 mlx 1	4 °C
Substrate	Powderx 1	4 °C
Dye Reagent	10 ml x 1	4 °C
Standard	Powderx 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at560 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Ethanol

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml ethanolto generate 5 mmol/L of standardstock solution, store at 4 °C for 3-4 monthsafter reconstitution.Then dilute to 0.5mmol/Lstandard top solutionby adding 100 µlstock solution into 900 µlethanol. Perform 2-fold serial dilutions of the top standardsolution using ethanolto make the standard curve. The concentration of standard curve could be 500/250/125/62.5/31.2/15.6/7.8µmol/L.

Substrate: Briefly centrifuge prior to opening. Add 2 ml Reaction Buffer to dissolve before use. Keep in dark and store at 4 °C for 1-2 weeks or -20°C for 3-4months after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use; store at -80°C for 1 monthafter reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For serum, plasma and other biological fluids samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Sample	10 µl	--	--	--	--
Positive Control	--	--	--	--	10 µl
Standard	--	--	10 µl	--	--
Distilled water	--	10 µl	20 µl	30 µl	--
Reaction Buffer	70 µl	70 µl	70 µl	70 µl	70 µl
Substrate	20 µl	20 µl	--	--	20 µl
Mix, then put it in the oven, incubate at37°C for 3 hours.					
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, record absorbance measured at 560 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit ofBeta-Glucuronidase activity is the enzyme thatproduce 1μmol ofthe Phenolphthalein acid per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times C_{Protein}\times T}(U/mg)$$

2.3 According to the weight of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample}/ V_{Assay})\times T}(U/g)$$

2.4 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

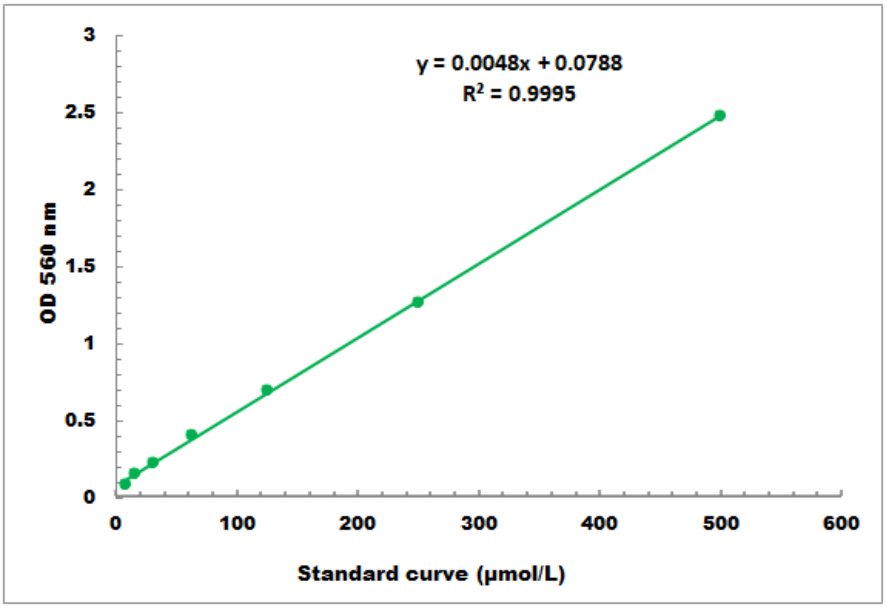
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

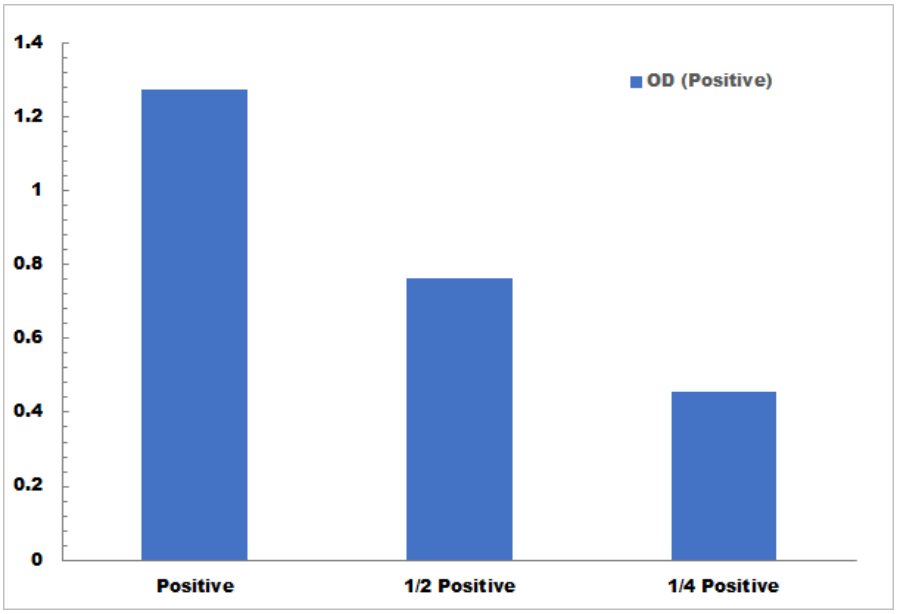
T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 5µmol/L - 500 µmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration