Beta-Glucuronidase

Microplate Assay Kit

User Manual

Catalog # CAK1167

(Version 1.3B)

Detection and Quantification of Beta-Glucuronidase (GGT) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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-Glucuronidase Microplate Assay Kit is designed to measure mannitol in various samples in 96-well microplate. The color intensity at 560nm is directly proportional to beta-Glucuronidase activity in the sample.

II. KIT COMPONENTS

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

Substrate: add 2 ml Reaction Buffer to dissolve before use; store at -20 °C for 1 month after reconstitution.

Standard: add 1 ml ethanol to dissolve before use, then add 100 µl in to 900 µl ethanol, the concentration will be 500 µmol/L; store at -20 °C for 1 month after reconstitution.

Positive Control: add 1 ml Assay Buffer to dissolve before use; store at -80 °C for 1 month after reconstitution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 560 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Ethanol

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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.

V. 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 at 37 °C for 3 hours.					
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, record absorbance measured at 560 nm.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of Beta-Glucuronidase activity is the enzyme that produce 1 μmol of the Phenolphthalein acid per minute.

1. According to the protein concentration of sample

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

$$= 0.00278 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.00278 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

$$\text{Beta-GD (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.00278 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of standard, 500 μmol/L = 0.5 μmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

V_{Sample}: the volume of sample, 0.01 ml;

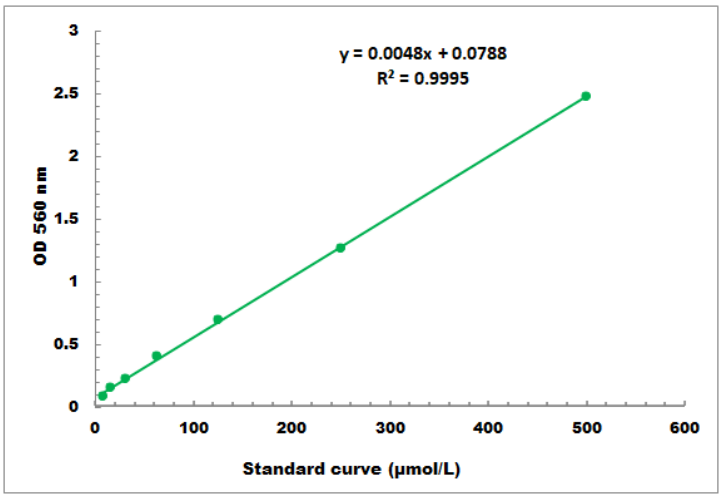
$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

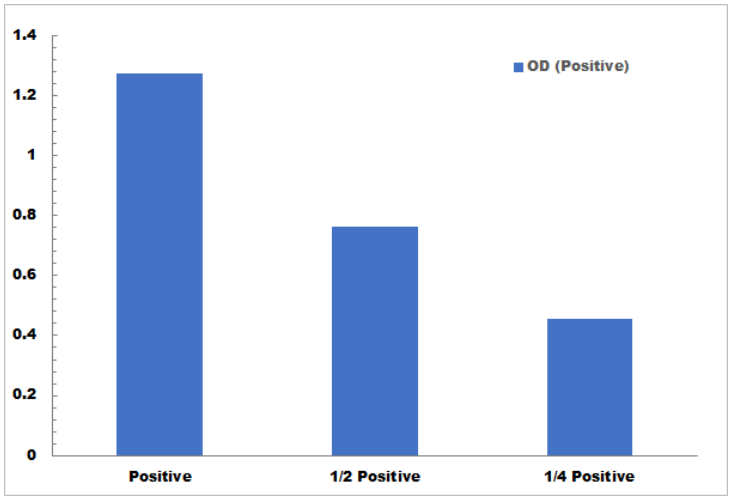
T: the reaction time, 3 hours = 180 minutes.

VII. TYPICAL DATA

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



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



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES