Beta-Glucuronidase

Microplate Assay Kit

Cat #: orb707343 (manual)

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.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

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.

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	
Technical Manual	1 Manual	

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.

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

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.

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.

CALCULATION

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

1. According to the protein concentration of sample

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

$$= 166.67 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 166.67 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

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

$$= 166.67 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

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

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

W: the weight of sample, g;

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

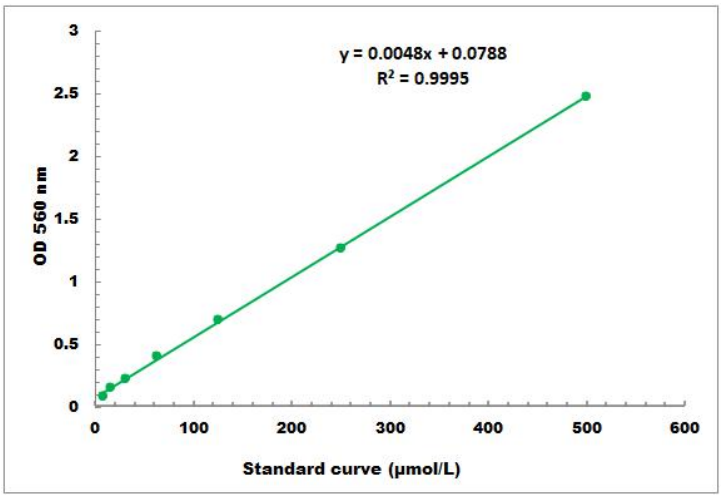
$V_{Standard}$: the volume of standard, 0.01 ml;

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

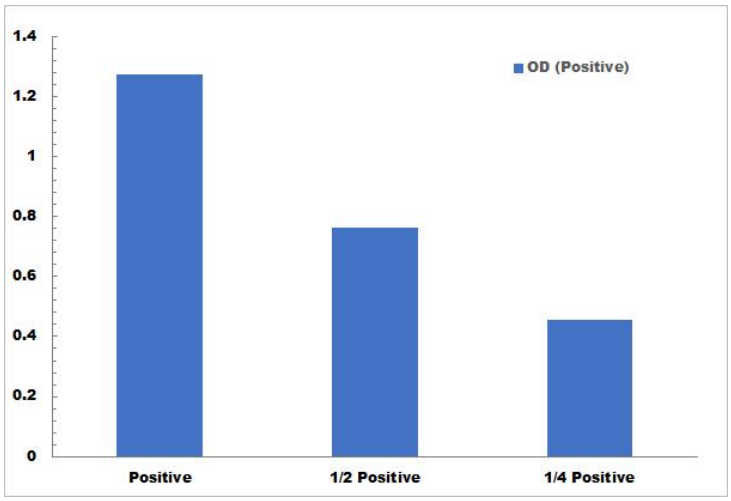
T: the reaction time, 3 hours.

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

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558