Beta-Glucosidase Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6704

Detection and Quantification of Beta-Glucosidase (β-GC)Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

1. Introduction

β-Glucosidase is a glucosidase enzyme which acts upon β1 ->4 bonds linking two glucose or glucose-substituted molecules. β -Glucosidases are required by organisms (some fungi, bacteria, termites) for consumption of cellulose. Lysozyme is also a β-glucosidase and is present in tears to prevent bacterial infection of the eye. In humans, lower activity of a β -glucosidase isoform (lysosomal gluco-cerebrosidase) has been related to Gaucher's disease and Parkinson' s disease.

Beta-Glucosidase ActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring Beta-Glucosidase activity in a variety of samples. The assay is initiated with the enzymatic hydrolysis of the glucoside byβ-Glucosidase. The enzyme catalysed reaction product sp-nitrophenol , can be measured at a colorimetric readout at 405 nm.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	5 mL x 1	4 °C
Substrate	Powder x 1	-20 °C
Dye Reagent	15 mL x 1	4 °C
Standard (1mmol/L)	1 mL x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor , multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening.Perform 2 -fold serial dilutions of the top standardsolution using distilled water to make the standard curve. The concentration of standard curve could be 1000/500/250/125/62.5/31.25/15.625μmol/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 3-6months after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 1 mL distilled water to dissolve before use, then add 0.25 mL into 0.75 mL distilled water , mix.Store at -80°C for 1 month after reconstitution.

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

5. Sample Preparation

1. For cell and bacteria samples Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mL Assay Buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s ,repeat 30 times); centrifuged at 1 0,000g 4°C for 2 0 minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples Weigh out 0.1 g tissue, homogenize with 1 mL Assay Buffer on ice, centrifuged at 10,000g 4°C for 2 0 minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

6. Assay Procedure

Add following reagents in to the microplate :

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Sample	10 μl	--	--	--	--
Distilled water	--	10 μl	--	--	--
Positive Control	--	--	--	--	10 μl
Substrate	20 μl	20 μl	--	--	20 μl
Reaction Buffer	20 μl	20 μl	--	--	20 μl
Mix, put it in the oven, 37°C for 30 minutes.					
Standard	--	--	50 μl	--	--
Dye Reagent	150 μl	150 μl	150 μl	200 μl	150 μl
Mix, record absorbance measured at 405 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.

7. Calculation

Unit Definition:

One unit of β-Glucosidase activity is defined as the enzyme generates

1μmol of p-nitrophenol per minute .

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \ (U/10^4)$$

2.3 According to the weight of sample

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

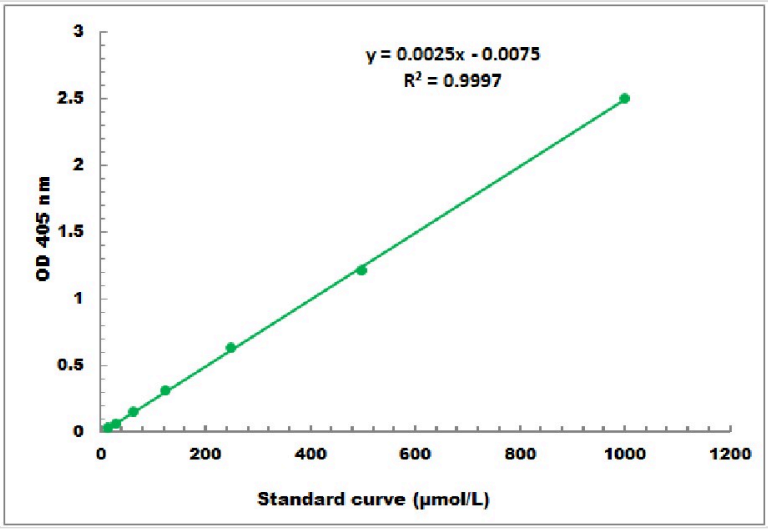
2.4 According to the volume of sample

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

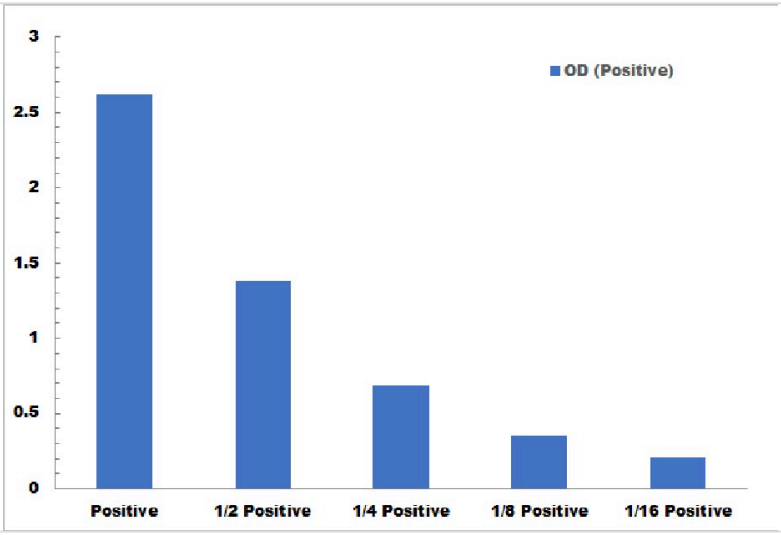
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Protein}$	the protein concentration of sample, mg/mL
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, μmol/ L
$V_{Standard}$	the volume 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

8. Typical Data

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



Detection Range : 10μmol /L -1000μmol/L



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