Alpha-Glucosidase Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6703

Detection and Quantification of Alpha-Glucosidase (α-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

α-Glucosidase breaks down α -1,4 linked polysaccharides to glucose, which can be utilized as a source of energy. In the biotechnology industry, α -glucosidase is used to produce glucose from intermediate breakdown products of starch hydrolysis generated by enzymes such as amylase. Pompe disease, one of the 12 known glycogen storage diseases, is an autosomal recessive metabolic disorder attributed to α-glucosidase deficiency. In this disease, glycogen accumulates in the lysosomes, resulting in progressive muscle weakness, heart failure and other neurological symptoms.

Alpha-Glucosidase Activity Colorimetric Microplate Assay Kit is initiated with the enzymatic hydrolysis of the glucoside by α-Glucosidase. The enzyme catalysed reaction products p-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 mL x 4	4 °C
Reaction Buffer	5 mL x 1	4 °C
Substrate	Powder x 1	-20 °C, keep in dark
Dye Reagent	15 mL x 1	4 °C
Standard (1 mmol/L)	1 mL x 1	-20 °C, keep in dark
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 standard solution using distilled water to make the standard curve. The concentration of standard curve could be 1000/500/250/125/62.5/31.2 /15.6 μmol/ L.

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

Positive Control: Briefly centrifuge prior to opening. Dissolve in 1 mL distilled water , then add 0.25 mL into 0.75 mL distilled water. Store at -20°C for 1 months.

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

Note:

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

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

7. Calculation

Unit Definition:

One unit of α-Glucosidase activity is defined as the enzyme generates 1 μmol of p-nitrophenol per minute .

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2 According to the quantity of cells or bacteria

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

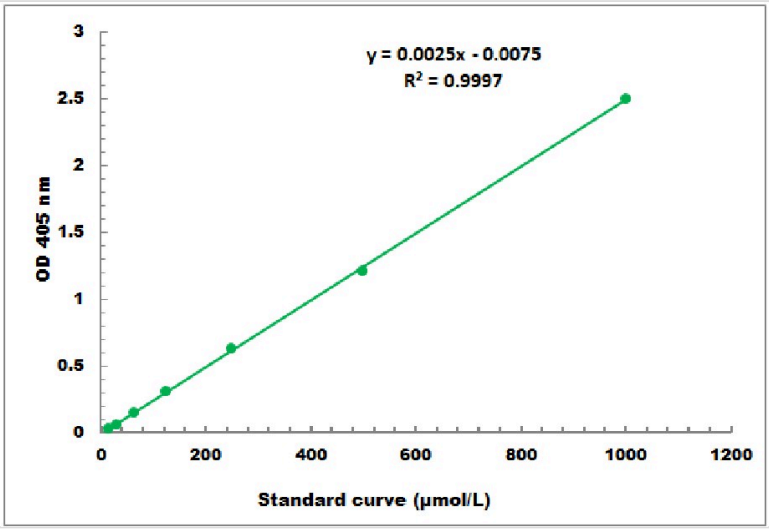
2.3 According to the weight of sample

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

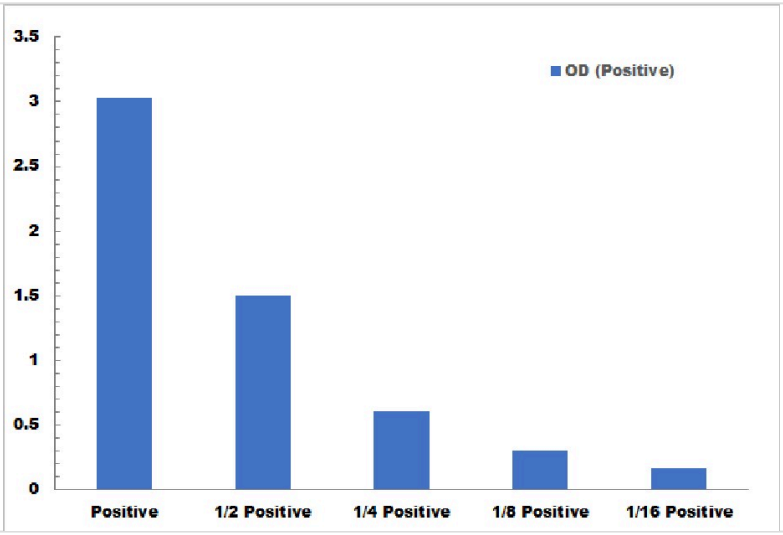
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Protein}$	the protein concentration of sample, mg/mL
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, μmol/mL
$V_{Standard}$	the volume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

8. Typical Data

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



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



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