Alpha-Glucosidase Microplate Assay Kit User Manual

Catalog # ASK1096

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.

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I. 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.

The assay 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.

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II. 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
Stop Solution	15 ml x 1	4 °C
Standard (1 mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: Add 2 ml Reaction Buffer to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

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

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IV. 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%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000g 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 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

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V. ASSAY PROCEDURE

Add following reagents into the microplate:

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

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VI. CALCULATION

Unit Definition: One unit of α-Glucosidase activity is defined as the enzyme that generates 1 μmol of p-nitrophenol per hour.

1. According to the protein concentration of sample

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

$$= 10 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 10 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

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

$$= 10 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

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

$C_{Standard}$: the concentration of Standard, 1 mmol/L = 1 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

$V_{Standard}$: the volume of standard, 0.05 ml;

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

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

T: the reaction time, 0.5 hour.

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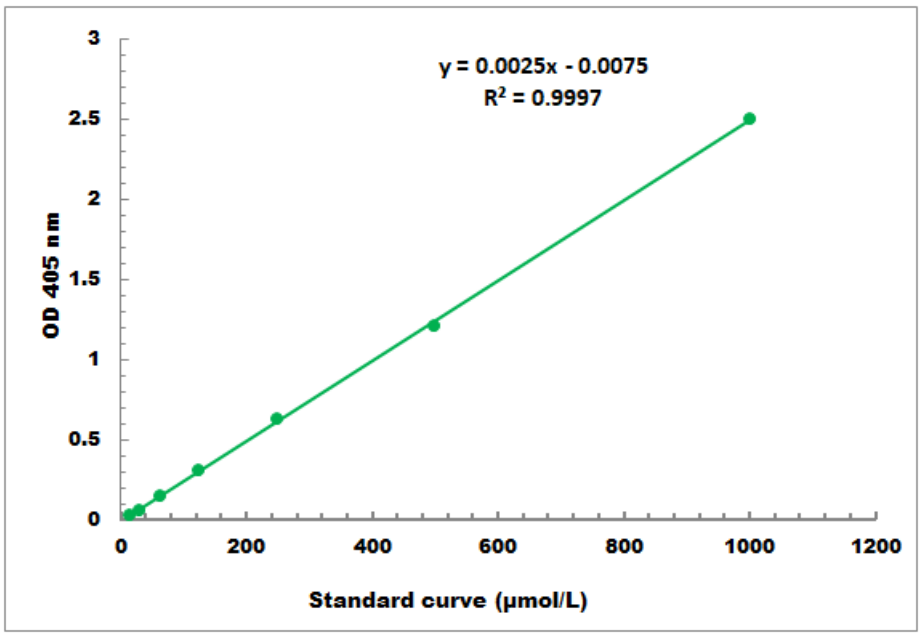
VII. TYPICAL DATA

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



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

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