Glucoamylase Microplate Assay Kit

User Manual

Catalog # CAK1241

(Version 1.2A)

Detection and Quantification of Glucoamylase Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Glucoamylase is an enzyme that can be obtained from the yeast S. diastaticus or fungi in the Aspergillus genus such as Aspergillus niger. The enzyme decomposes starch molecules in the human body into the useful energy compound of glucose. This is accomplished by removing the alpha-1 and 4-glycosidic linkages from the non-reducing end of the starch molecule. These molecules are more commonly referred to as polysaccharides and are frequently either amylase- or amylopectin-based. The purpose of glucoamylase in commercial food activities is centered around the brewing of beer and the production of bread products and fruit juices.

Glucoamylase Microplate Assay Kit is a sensitive assay for determining Glucoamylase activity in various samples. The enzyme catalysed reaction products react with 3,5-dinitrosalicylic acid. The intensity of the product color, measured at 540 nm, is proportional to the Glucoamylase activity in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	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	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 9 ml Assay Buffer to dissolve before use, mix, heat in boiling water bath for 1 minute.

Standard: add 1 ml distilled water to dissolve before use; then add 0.3 ml into 0.7 ml distilled water, the concentration will be 3 mmol/L.

Positive Control: add 1 ml Assay Buffer to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

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%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4 °C for 10 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 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly, or dilute with Assay Buffer.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Blank	Standard	Positive Control
Substrate	90 µl	--	--	90 µl
Sample	10 µl	--	--	--
Distilled water	--	100 µl	--	--
Positive Control	--	--	--	10 µl
Mix, cover the plate adhesive strip, put the plate into the convection oven, incubate at 40 °C for 10 minutes.				
Standard	--	--	100 µl	--
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, cover the plate adhesive strip, put the plate into the convection oven, 90 °C for 10 minutes. When cold, record absorbance measured at 540 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 glucoamylase activity is defined as the enzyme generates 1 μmol of reducing sugars per minute.

1. According to the protein concentration of sample

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

$$= 3 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 3 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

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

$$= 3 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

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

$$= 3 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the standard concentration, 3 mmol/L = 3 μmol/ml;

W: the weight of sample, g;

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

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

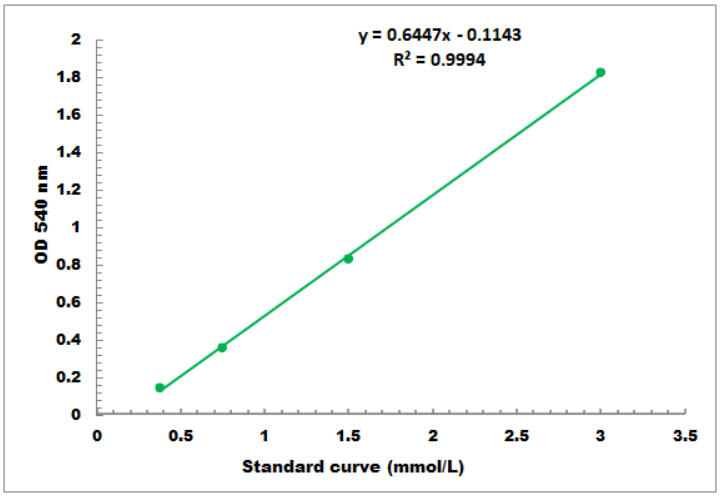
$V_{Standard}$: the volume of standard, 0.1 ml;

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

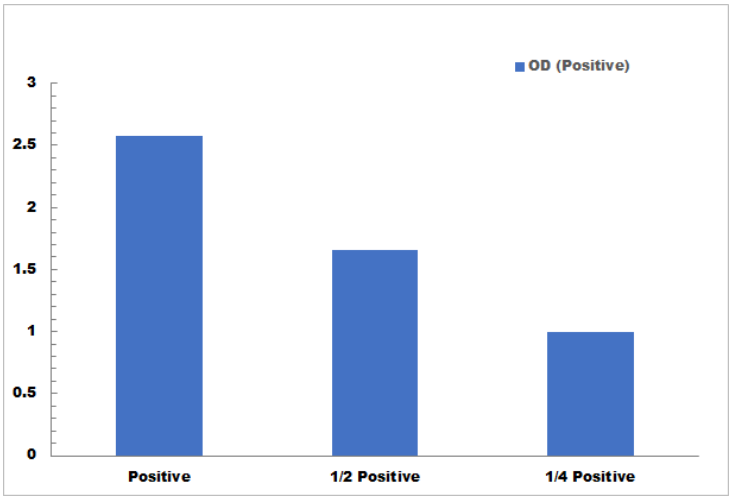
T: the reaction time, 10 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.3 mmol/L - 3 mmol/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