



# **Glucoamylase Microplate Assay Kit**

## **User Manual**

**Catalog # FTA0218**

(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.**

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## 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 0.2 ml into 0.8 ml distilled water, the concentration will be 5 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  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonication 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 $\mu$ l	--	--	90 $\mu$ l
Sample	10 $\mu$ l	--	--	--
Distilled water	--	100 $\mu$ l	--	--
Positive Control	--	--	--	10 $\mu$ l
Mix, cover the plate adhesive strip, put the plate into the convection oven, incubate at 40 °C for 10 minutes.				
Standard	--	--	100 $\mu$ l	--
Dye Reagent	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
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.

## VI. CALCULATION

**Unit Definition:** One unit of Glucoamylase activity is the enzyme that generates 1  $\mu\text{mol}$  of reducing sugars per minute.

### 1. According to the protein concentration of sample

$$\begin{aligned}\text{Glucoamylase (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \\ &\quad / (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 5 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

### 2. According to the weight of sample

$$\begin{aligned}\text{Glucoamylase (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \\ &\quad / (W \times V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 5 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W\end{aligned}$$

### 3. According to the quantity of cell or bacteria

$$\begin{aligned}\text{Glucoamylase (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \\ &\quad / (N \times V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 5 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / N\end{aligned}$$

### 4. According to the volume of sample

$$\begin{aligned}\text{Glucoamylase (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \\ &\quad / V_{\text{Sample}} / T \\ &= 5 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}})\end{aligned}$$

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

$C_{\text{Standard}}$ : the standard concentration, 5 mmol/L = 5  $\mu\text{mol/ml}$ ;

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

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

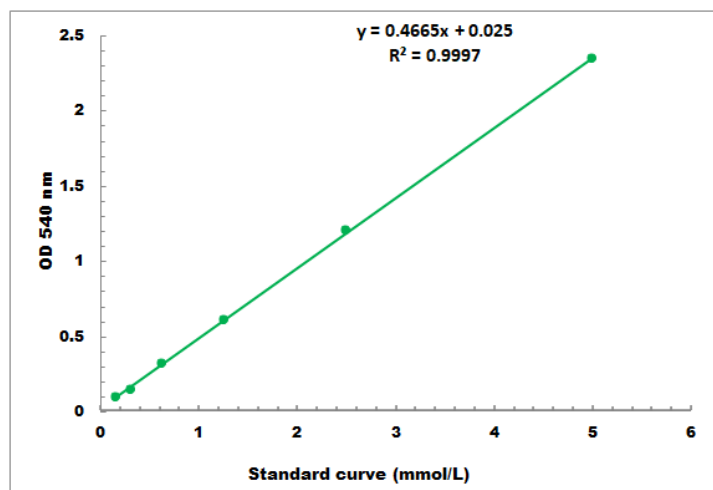
$V_{\text{Standard}}$ : the volume of standard, 0.1 ml;

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

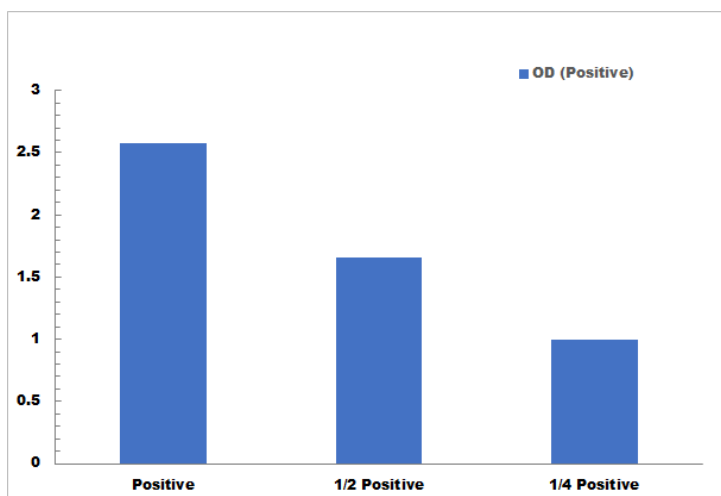
T: the reaction time, 10 minutes.

## VII. TYPICAL DATA

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



Detection Range: 0.1 mmol/L - 5 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](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES