Glucoamylase Assay Kit

ARG83422 Glucoamylase Assay Kit can be used to measure Glucoamylase in tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83422

Package: 96 wells

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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.

PRINCIPLE OF THE ASSAY

Glucoamylase Assay Kit determined Glucoamylase activity in Tissue extracts, cell lysate, cell culture media and other biological fluids. The enzyme catalysed reaction products react with 3,5-dinitrosalicylic acid. The Glucoamylase activity can be measured at a colorimetric readout at 540 nm

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Positive Control	1 vial (lyophilized)	4°C
Assay Buffer	4 X 30 ml	4°C
Substrate	1 vial (lyophilized)	4°C
Reagent Dye	10 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store all component at 4°C.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and bacteria - 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

Tissue - 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.

Note: For liquid sample, it can be assayed directly.

REAGENT PREPARATION

- **Standard:** Reconstitute the Standard with **1 ml** of **distilled water.** Allow the Standard keep on bench for few minutes. Make sure the Standard is dissolved completely. Add **0.3 ml** of **Standard** into **0.7 ml** of **distilled water**, the concentration will be 3 **μmol/mL**.
- **Substrate:** Reconstitute the Substrate with **9 ml** of **Assay Buffer**. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.
- **Positive Control:** Reconstitute the Positive Control with **1 ml** of **Assay Buffer**. Allow the Positive Control keep on bench for few minutes. Make sure the Positive Control is dissolved completely and mixed thoroughly before use.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **90 µl** of **Substrate** into Sample and Positive Control wells.
2. Add **10 µl** of **Sample** into Sample wells.
3. Add **10 µl** of **Positive Control** into Positive Control wells.
4. Mix well. Incubate at **40°C** for **10 min**.
5. Add **100 µl** of **Standard** into Standard wells.
6. Add **100 µl** of **Reagent Dye** into each wells.
7. Mix well. Incubate at **90°C** for **10 min**. Read the OD at **540 nm**.

Summary of Glucoamylase Assay Procedure

Reagent	Standard	Sample	Positive Control	Blank
Substrate	-	90 µl	90 µl	-
Sample	-	10 µl	-	-
Positive Control	-	-	10 µl	-
Distilled water	-	-	-	100 µl
Mix well. Incubate at 40°C for 10 min				
Standard	100 µl	-	-	-
Substrate II	100 µl	100 µl	100 µl	100 µl
Mix well. Incubate at 90°C for 10 min. Read the OD at 540 nm.				

CALCULATION OF RESULTS

1. Unit Definition: One unit Glucoamylase activity is defined as generates 1 μmol of reducing sugar per minute in the reaction system.

2. Calculate the average absorbance values for each set of samples and control.

3. Calculation:

A. Definition:

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

$C_{Standard}$: the concentration of Standard, 3 μmol/ml;

V_{Sample}: the volume of reaction sample, 10 μl = 0.01 ml;

$V_{Standard}$: the volume of reaction Standard, 100 μl = 0.1 ml;

V_{assay}: the volume of Assay Buffer, 1000 μl = 1 ml;

W: the weight of sample, g;

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

T: the reaction time, 10 minutes.

B. Formula:

a). According to the protein concentration of sample

Glucoamylase activity (U/mg) =

$(OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T]$

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

b). According to the weight of sample

Glucoamylase activity (U/mg) =

$(OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{Assay}) \times T]$

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

c). According to the cell or bacteria

Glucoamylase activity ($U/10^4$) =

$(OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (N \times V_{Sample} / V_{Assay}) \times T]$

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

d). According to the volume of sample

Glucoamylase activity (U/ml) =

$(OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times T]$

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