



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

For research use only. Not for use in diagnostic procedures.

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Tel: +886-3-6221320

Fax: +886-3-5530266

Email: info@arigobio.com

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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
Plate Adhesive Strips	3 Strips	

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 $\mu\text{mol/mL}$.
- **Substrate:** Reconstitute the Substrate with **9 ml of Assay Buffer** to dissolve before use, mix, heat in boiling water bath for 1 minute. 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.

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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. Add **100 µl** of **Distilled water** into Blank wells.
5. Mix, cover the plate adhesive strip, put the plate into the convection oven, incubate at **40°C** for **10 min**.
6. Add **100 µl** of **Standard** into Standard wells.
7. Add **100 µl** of **Reagent Dye** into each wells.
8. Mix, cover the plate adhesive strip, put the plate into the convection oven, **90°C** for **10 min**. When cold, 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, cover the plate adhesive strip, put the plate into the convection oven, incubate at 40°C for 10 min				
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 min . When cold, read the OD at 540 nm .				

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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.

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, $3 \mu\text{mol/ml}$;
 - V_{Sample} : the volume of reaction sample, $10 \mu\text{l} = 0.01 \text{ ml}$;
 - V_{Standard} : the volume of reaction Standard, $100 \mu\text{l} = 0.1 \text{ ml}$;
 - V_{Assay} : the volume of Assay Buffer, $1000 \mu\text{l} = 1 \text{ ml}$;
 - W : the weight of sample, g ;

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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) =

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

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

b). According to the weight of sample

Glucoamylase activity (U/mg) =

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

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

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c). According to the cell or bacteria

Glucoamylase activity (U/10⁴) =

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

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

d). According to the volume of sample

Glucoamylase activity (U/ml) =

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

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