alpha Glucosidase Activity Assay Kit (Colorimetric)

alpha Glucosidase Activity Assay Kit (Colorimetric) can be used to measure alpha Glucosidase activity in biological samples.

Catalog number: ARG82166

Package: 100 tests

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INTRODUCTION

Alpha-glucosidase (EC 3.2.1.20, maltase, glucoinvertase, glucosidosucrase, maltase-glucoamylase, alpha-glucopyranosidase, glucosidoinvertase, alpha-D-glucosidase, alpha-glucoside hydrolase, alpha-1,4-glucosidase, alpha-D-glucoside glucohydrolase) is a glucosidase located in the brush border of the small intestine that acts upon α(1→4) bonds. This is in contrast to beta-glucosidase. Alpha-glucosidase breaks down starch and disaccharides to glucose. Maltase, a similar enzyme that cleaves maltose, is nearly functionally equivalent. [Provide by Wikipedia: Alpha-glucosidase]

PRINCIPLE OF THE ASSAY

This alpha Glucosidase Activity Assay Kit (Colorimetric) is a simple assay that measures the amount of alpha Glucosidase present in biological samples. This assay is designed to measure α-glucosidase activity directly in biological samples without pretreatment. The improved method utilizes p-nitrophenyl-α-D-glucopyranoside that is hydrolyzed specifically by α-glucosidase into a yellow colored product (maximal absorbance at O.D. 405 nm). The rate of the reaction is directly proportional to the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer (pH 7.0)	24 mL	-20°C
α-NPG Substrate	1 mL	-20°C
Standard (250 U/L)	10 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 405 nm
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The following chemicals are known to affect the enzyme activity and should be avoided. SH-containing reagents (E.g., dithiothreitol, 2-mercaptoethanol, glutathione), Ca^{2+}, Cu^{2+}, Fe^{3+}/Fe^{2+}, Hg^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+}, SDS, Triton X-100, Tween, digitonin, EDTA and Tris.
- This assay is based on a kinetic reaction. Use of a multi-channel pipettor is recommended. Addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Assays can be executed at room temperature or 37°C.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- 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 assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Other liquid biological sample: Assay directly.

Note:

- The following chemicals are known to affect the enzyme activity and should be avoided. SH-containing reagents (E.g., dithiothreitol, 2-mercaptoethanol, glutathione), Ca^{2+}, Cu^{2+}, Fe^{3+}/Fe^{2+}, Hg^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+}, SDS, Triton X-100, Tween, digitonin, EDTA and Tris.

REAGENT PREPARATION

- **Working Reagent:** for each well, mixing 8 µL of α-NPD Substrate and 200 µL of Assay Buffer. Fresh reconstitution is recommended. The Working Reagent is stable for at least one day at room temperature.

ASSAY PROCEDURE

Equilibrate reagents to room temperature or 37°C. Briefly centrifuge tubes before use.

	Standard well	Blank well	Sample well
Standard	200 µL		
Distilled water	20 µL	220 µL	
Samples			20 µL
Working Reagent			200 µL
Tap plate to mix briefly. Incubate for **0 and 20 minutes** at **room temperature** or **37°C**.			
Read the absorbance at **O.D. 405 nm** at **0 and 20 minutes.**			

Note: This assay is based on a kinetic reaction. Use of a multi-channel pipettor is recommended. Addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Assays can be executed at room temperature or 37°C.

CALCULATION OF RESULTS

1. Calculate sample alpha glucosidase activity using the equation,

   α-Glucosidase (U/L) = $[(OD_{20mins} - OD_{0mins}) / (OD_{STANDARD} - OD_{DITILLEDWATER})]$ x 250

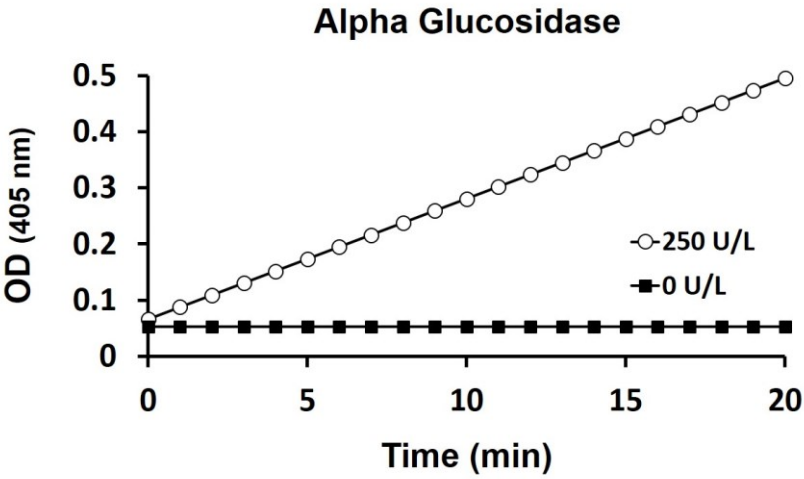
   Note:

   - OD_{20mins}, OD_{0mins}: the O.D. 405 nm values at 20 minutes and 0 minutes for the sample
   - $OD_{STANDARD}$, $OD_{DITILLEDWATER}$: OD405 nm values of the Standard and distilled water blank at 20 minutes.
   - The value 250 is the equivalent activity (U/L) of the Standard under the assay conditions.

2. Unit definition: one unit of enzyme catalyzes the hydrolysis of 1 μmole of substrate per min at pH 7.0.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the alpha Glucosidase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

2 U/L