Tribioscience

α-Glucosidase Activity Assay (TBS2208, 100 Assays, Store at -20°C)

DESCRIPTION

α-Glucosidase (α-GC), also known as α-D-glucoside hydrolase is a crucial enzyme for carbohydrate digestion. It can convert complex sugars into glucose with significant roles in metabolism, diabetes treatment, and drug discovery.

Tribioscience α-Glucosidase Activity Colorimetric Assay provides a simple and sensitive method for monitoring α-glucosidase activity in biological samples (tissue, cells, serum, urine, stool). This assay uses a synthetic p- α-Glucosidase substrate (p-NPG) to release p-nitrophenol (pNP), which turns yellow under alkaline conditions. The absorbance of pNP is measured at 405 nm as shown in **Fig. 1.**

Fig. 1: Assay Principle

Synonyms: Alpha Glucosidase; a glucosidase; α-GC; α-D-glucoside hydrolase.

APPLICATIONS

Determination of α-Glucosidase activity in biological samples.

KEY FEATURES

Flexible: can be used for 96 wells and 384 wells plate.
Simple: Just one-step: add-incubate-read model.
Time saving: a 30-minute reaction at 37˚C

KIT CONTENTS

Component	100x Rxns
α-Glucosidase Substrate (10x)	0.9 mL
pNP Standard (10mM)	0.1 mL
Positive control (10x)	5 µL
Assay Buffer	12 ml
Stop buffer	11 ml

STORAGE CONDITIONS

The kit is shipped on ice and should be stored at -20°C for shelf life of 12 months after receipt.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate and assay buffer into samples should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Sample Preparation: Serum and plasma can be assayed directly. For urine samples containing precipitation, centrifuge at 10,000 x g, 4°C for 3 minutes and assay the supernatant.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold PBS, approximately one million cells per mL. Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

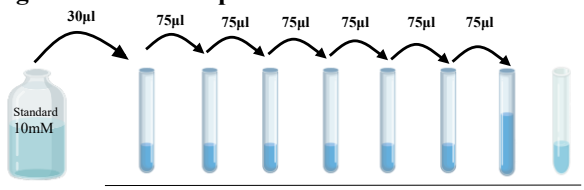
Reagent Preparation:

- Equilibrate all components to the room temperature. If substrate has precipitation in bottle, please warm up to dissolve the precipitation.
- Briefly vortex or pipette up and down all components (positive control can't be vortex) to ensure fresh reconstitution.

Reaction Preparation:

1. Label tubes as #1 through #8 as below table1.
2. Add 120 µL of Assay Buffer to Std1, and 75 µL to Std2 to 8.
3. Pipet 30 µL of 10 mM standard stock into Std#1. Then, make 2x series dilution in Std2 through 7 with addition of 75 µL. Std8 is 1x Assay Buffer alone **as a standard 0**. The standard concentration in tube 1 through 7 will be 2000, 1000, 500, 250, 125, 62.5, 31.25µM, **Tube#8 is Standard 0 as blank.**

Fig.2: Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	120	75	75	75	75	75	75	75
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	30	75	75	75	75	75	75	0
Final Conc (µM)	2000	1000	500	250	125	62.5	31.25	0

4. Add 45 ul of assay buffer to the 10x positive control.
5. Dilute substrate stock (10x) with assay buffer for 10 times. For 96 well plate: 9 mL Assay buffer + 1 mL substrate stock (10x). Mix well gently.
6. Transfer 20 µL of each sample, blank, positive control, and standards into two separate wells.
7. Add 80 µL of the substrate solution to all samples, positive control, and blank wells. Add 80 µL of Assay Buffer to each standard wells *(Note: Do not add substrate in the standard*). Tap plate briefly to mix.
8. Incubate at 37°C for 30 minutes. Add 100 ul of stop solution in each well.
9. Read plate at OD 405nm in the endpoint mode.

CALCULATION

Subtract blank OD (Standard 0, #8) from the standard OD values and plot the ΔOD against standard concentrations. Determine the slope, and use the following equation to calculate trypsin activity:

Glucosidase Activity (U/L) = DF * (ODSAMPLE – OD BLANK)/ (t * Slope)

where ODSAMPLE is the OD405nm value for each sample and ODBLANK is the OD405nm value of the sample blank. Slope is the linear regression fit of the standard points and t is the reaction time (30 min). DF is the dilution factor.

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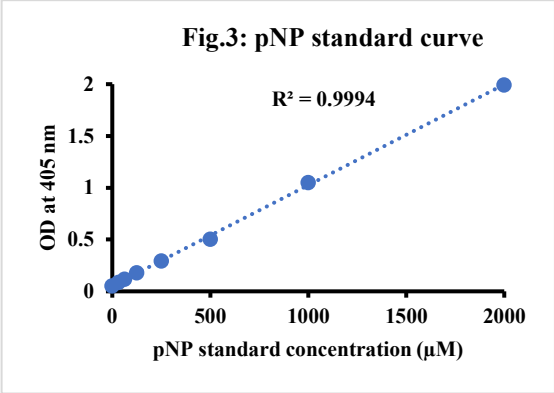
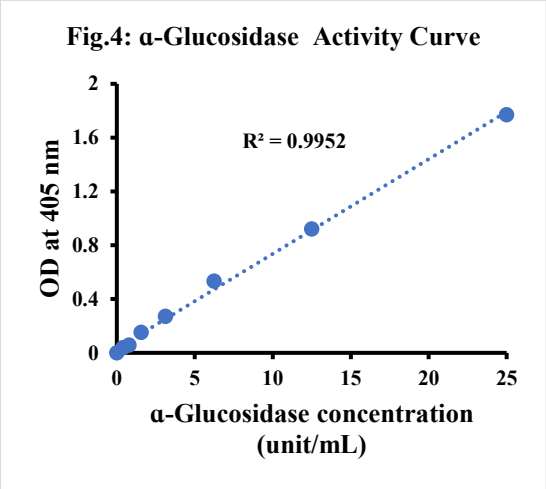
Unit definition: 1 Unit (U) will catalyze the conversion of 1 µmole of substrate to p-Nitrophenol per min at 37°C.

TYPICAL DATA

This standard curve is provided for demonstration only as **Fig.3.** A standard curve should be generated for each set of samples assayed.



TYPICAL DATA: Different concentrations of enzyme with substrate shown as Fig. 4.



RELATED PRODUCTS

Caspase-3 Fluorometric Assay kit (TBS3230)
Tryptase Activity Assay (TBS2101)
Cytochrome C Oxidase Activity Assay (TBS2115)
Fast Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
Glucose Oxidase Activity Colorimetric/Fluorometric Assay (TBS2088)
Non-esterified Fatty Acid Assy (TBS2203)
Glycerol Colorimetric / Fluorometric Assy (TBS2204)
Protein Assay Kits (TBS2005)
Cell Nuclear Extract kit (TBS6025)

Research use only.