α-Glucosidase Assay Kit (Colorimetric)

LS-K194-100 (100 Tests) • **Store at -20°C**

Introduction

α-GLUCOSIDASE hydrolyzes the terminal, non-reducing 1,4-linked Alpha-D-glucose residues with release of Alpha-D-glucose. Alpha-Glucosidase is needed by all animals to hydrolyze maltose to glucose for use as a food. Aberrant activities have been implicated in diseases such as diabetes and Pompe disease. Simple, direct and automation-ready procedures for measuring α-glucosidase activity are becoming popular in Research and Drug Discovery. This α-Glucosidase Assay Kit 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 405nm). The rate of the reaction is directly proportional to the enzyme activity.

Key Features

- High sensitivity and wide linear range. Use 20 µL sample. The detection limit is 2 U/L, linear up to 250 U/L.
- Homogeneous and simple procedure. Simple "mix-and-measure" procedure allows reliable quantitation of α-glucosidase activity within 20 minutes.
- Robust and amenable to HTS. All reagents are compatible with high-throughput liquid handling instruments.

Applications

- Direct Assays: α-glucosidase activity in biological samples.
- Characterization and Quality Control for α-glucosidase production.
- Drug Discovery: high-throughput screen and evaluation of α-glucosidase inhibitors.

Components

Component	K194-100 100 Tests
Assay Buffer (pH 7.0)	24 mL
α-NPG Substrate	1 mL
Calibrator (equivalent to 250 U/L)	10 mL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipettor). Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Storage

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

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Assay Procedure

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.

Reagent preparation

Equilibrate reagents to room temperature. The Working Reagent is prepared by mixing for each 96-well assay, 200 µL Assay Buffer and 8 µL α-NPG substrate (final 1.0 mM). Fresh reconstitution is recommended, although the Working Solution is stable for at least one day at room temperature.

Sample preparation

Enzyme samples can be in 50 mM phosphate (pH 7.0) buffer or in any other suitable enzyme buffer. 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.

Procedure using 96-well plate

1. Transfer 20 µL distilled water (H_2O) to two wells of a clear bottom 96-well plate. Add 200 µL H_2O to one of these wells and 200 µL Calibrator to the other well (total volume 220 µL).

   Transfer 20 µL samples into other wells. Transfer 200 µL Working Reagent to the sample wells only. The final reaction volume in the sample wells is 220 µL. Tap plate briefly to mix.

2. Read OD_{405nm} (t = 0), and again after 20 min (t = 20 min) on a plate reader.

Calculations

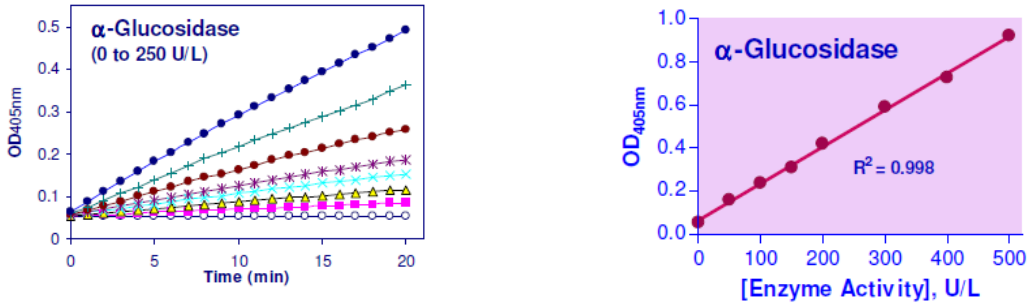
α-glucosidase activity of the sample (U/L) is

$$\alpha\text{-Glucosidase Activity} = \frac{OD_{20} - OD_0}{OD_{CALIBRATOR} - OD_{H2O}} \times 250\ (U/L)$$

OD_{20} and OD_0 are OD_{405nm} values of sample at 20 and 0 min, respectively. $OD_{CALIBRATOR}$ and OD_{H2O} are OD_{405nm} values of Calibrator and H_2O at 20 min.

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

Sample Data



Kinetics of α-glucosidase reaction in 96-well plate assay

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