QuantiChrom™ β-Glucosidase Assay Kit (DBGD-100)

Colorimetric Kinetic Determination of β-Glucosidase Activity

DESCRIPTION

β-GLUCOSIDASE is a glucosidase enzyme which acts upon β1->4 bonds linking two glucose or glucose-substituted molecules (i.e., the disaccharide cellobiose). β-Glucosidases are required by organisms (some fungi, bacteria, termites) for consumption of cellulose. Lysozyme is also a β-glucosidase and is present in tears to prevent bacterial infection of the eye. In humans, lower activity of a β-glucosidase isoform (lysosomal gluco-cerebrosidase) has been related to Gaucher's disease and Parkinson's disease.

Simple, direct and automation-ready procedures for measuring β-glucosidase activity are becoming popular in Research and Drug Discovery. BioAssay Systems' QuantiChrom™ β-Glucosidase Assay Kit is designed to measure β-glucosidase activity directly in biological samples without pretreatment. The improved method utilizes *p*-nitrophenyl-β-D-glucopyranoside that is hydrolyzed specifically by β-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 β-glucosidase activity within 20 minutes.

Robust and amenable to HTS. All reagents are compatible with high-throughput liquid handling instruments.

APPLICATIONS

Direct Assays: β-glucosidase activity in biological samples.

Characterization and Quality Control for β-glucosidase production.

Drug Discovery: high-throughput screen for β-glucosidase modulators.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer: 24 mL (pH 7.0)

β-NPG Substrate: 1 mL

Calibrator: 10 mL (equivalent to 250 U/L)

Storage conditions. The kit is shipped at room temperature. Store all components at -20℃. Shelf life of 6 months after receipt.

Precautions: 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.

PROCEDURES.

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 Solution is prepared by mixing for each 96-well assay, 200 µL Assay Buffer and 8 µL β-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, 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.

4. *Calculation*: β-glucosidase activity of the sample (U/L) is

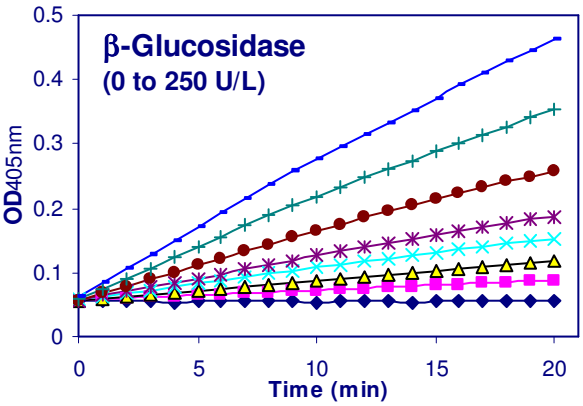
$$\text{β-Glucosidase Activity} = \frac{OD_{20} - OD_0}{OD_{CALIBRATOR} - OD_{H2O}} \times 250 \text{ (U/L)}$$

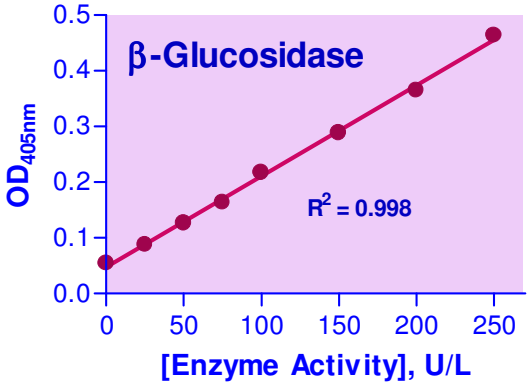
OD_{20} and OD_0 are OD_{405nm} values of sample at 20 and 0 min, respectively. $OD_{CALIBRATOR}$ and OD_{H2O} are OD_{405nm} values of Calibrator and H_2O at 20 min.

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

MATERIALS REQUIRED, BUT NOT PROVIDED

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





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

LITERATURE

[1]. Bhat, M.K. et al. (1993). Purification and characterization of an extracellular β-glucosidase from the thermophilic fungus Sporotrichum thermophile and its influence on cellulase activity. *Journal of General Microbiology* 139: 2825-2832.

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[3]. IWASHITA, K. et al. (1998) Purification and characterization of extracellular and cell wall bound β-glucosidases from Aspergillus kawachii. *Bioscience, Biotechn. Biochem.* 62 (10): 1938-1946.