



# beta-Glucosidase Assay Kit

Catalog Number KA1611

100 assays

Version: 03

Intended for research use only

[www.abnova.com](http://www.abnova.com)

## Table of Contents

<b>Introduction .....</b>	<b>3</b>
Intended Use .....	3
Background .....	3
<b>General Information .....</b>	<b>4</b>
Materials Supplied .....	4
Materials Required but Not Supplied .....	4
Storage Instruction .....	4
Precautions for Use .....	4
<b>Assay Protocol .....</b>	<b>5</b>
Reagent Preparation .....	5
Sample Preparation .....	5
Assay Procedure .....	5
<b>Data Analysis .....</b>	<b>6</b>
Calculation of Results .....	6
<b>Resources .....</b>	<b>7</b>
References .....	7

## Introduction

### Intended Use

#### Application

- ✓ Direct Assays:  $\beta$ -glucosidase activity in biological samples.
- ✓ Characterization and Quality Control for  $\beta$ -glucosidase production.
- ✓ Drug Discovery: high-throughput screen for  $\beta$ -glucosidase modulators

#### Features

- ✓ High sensitivity and wide linear range: Use 20  $\mu$ L 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  $\beta$ -glucosidase activity within 20 minutes.
- ✓ Robust and amenable to HTS: All reagents are compatible with high-throughput liquid handling instruments.

### Background

$\beta$ -GLUCOSIDASE is a glucosidase enzyme which acts upon  $\beta$ 1- $\rightarrow$ 4 bonds linking two glucose or glucose-substituted molecules (i.e., the disaccharide cellobiose).  $\beta$ -Glucosidases are required by organisms (some fungi, bacteria, termites) for consumption of cellulose. Lysozyme is also a  $\beta$ -glucosidase and is present in tears to prevent bacterial infection of the eye. In humans, lower activity of a  $\beta$ -glucosidase isoform (lysosomal gluco-cerebrosidase) has been related to Gaucher's disease and Parkinson's disease.

Simple, direct and automation-ready procedures for measuring  $\beta$ -glucosidase activity are becoming popular in Research and Drug Discovery. Beta-Glucosidase Assay Kit is designed to measure  $\beta$ -glucosidase activity directly in biological samples without pretreatment. The improved method utilizes p-nitrophenyl- $\beta$ -D-glucopyranoside that is hydrolyzed specifically by  $\beta$ -glucosidase into a yellow colored product (maximal absorbance at 405nm). The rate of the reaction is directly proportional to the enzyme activity

## General Information

### Materials Supplied

List of component

Component	Amount
Assay Buffer: (pH 7.0)	24 mL
$\beta$ -NPG Substrate	1 mL
Calibrator: (equivalent to 250 U/L)	10 mL

### Materials Required but Not Supplied

Pipetting devices and accessories.

Procedure using 96-well plate:

Clear flat-bottom 96-well plates and plate reader.

### Storage Instruction

Store all reagents at -20°C. Shelf life: 6 months after receipt.

### Precautions for Use

- Precautions

Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

## **Assay Protocol**

### **Reagent Preparation**

Equilibrate reagents to room temperature. The Working Solution is prepared by mixing for each 96-well assay, 200  $\mu\text{L}$  Assay Buffer and 8  $\mu\text{L}$   $\beta$ -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),  $\text{Ca}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}/\text{Fe}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , SDS, EDTA and Tris

### **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.

Procedure using 96-well plate:

1. Transfer 20  $\mu\text{L}$  distilled water ( $\text{H}_2\text{O}$ ) to two wells of a clear bottom 96-well plate. Add 200  $\mu\text{L}$   $\text{H}_2\text{O}$  to one of these wells and 200  $\mu\text{L}$  Calibrator to the other well (total volume 220  $\mu\text{L}$ ).  
Transfer 20  $\mu\text{L}$  samples into other wells. Transfer 200  $\mu\text{L}$  Working Reagent to the sample wells only. The final reaction volume in the sample wells is 220  $\mu\text{L}$ . Tap plate briefly to mix.
2. Read OD405nm ( $t = 0$ ), and again after 20 min ( $t = 20$  min) on a plate reader.

## Data Analysis

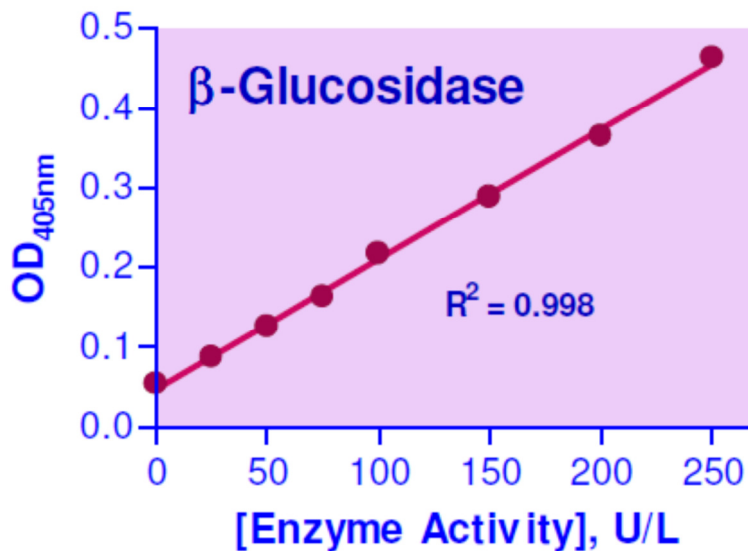
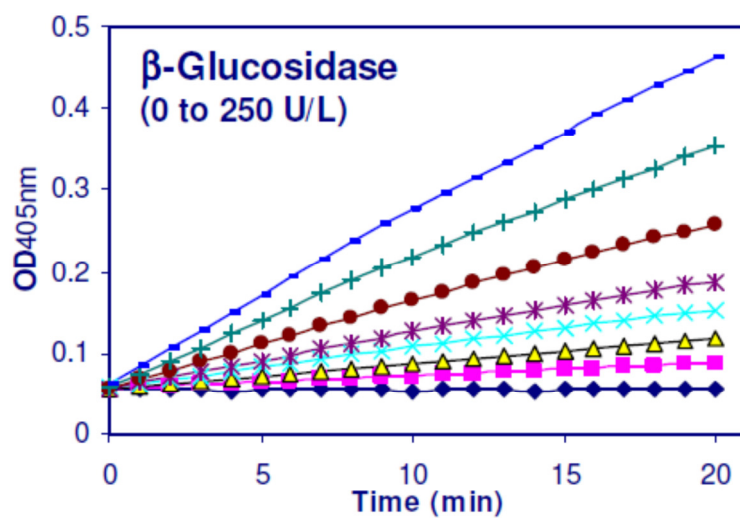
### Calculation of Results

Calculation:  $\beta$ -glucosidase activity of the sample (U/L) is

$$\beta\text{-Glucosidase Activity} = \frac{OD_{20} - OD_0}{OD_{\text{CALIBRATOR}} - OD_{\text{H}_2\text{O}}} \times 250 \text{ (U/L)}$$

$OD_{20}$  and  $OD_0$  are  $OD_{405\text{nm}}$  values of sample at 20 and 0 min, respectively.  $OD_{\text{CALIBRATOR}}$  and  $OD_{\text{H}_2\text{O}}$  are  $OD_{405\text{nm}}$  values of Calibrator and  $\text{H}_2\text{O}$  at 20 min.

Unit definition: one unit of enzyme catalyzes the hydrolysis of 1  $\mu\text{mole}$  of substrate per min at pH 7.0.



Kinetics of  $\beta$ -glucosidase reaction in 96-well plate assay

## Resources

### References

- ✓ Bhat, M.K. et al. (1993). Purification and characterization of an extracellular  $\beta$ -glucosidase from the thermophilic fungus *Sporotrichum thermophile* and its influence on cellulase activity. *Journal of General Microbiology* 139: 2825-2832.
- ✓ Kaur, J. et al (2007). Purification and characterization of  $\beta$ -glucosidase from *Melanocarpus* sp. *Electronic J. Biotechn.* 10: 260-270.
- ✓ IWASHITA, K. et al. (1998) Purification and characterization of extracellular and cell wall bound  $\beta$ -glucosidases from *Aspergillus kawachii*. *Bioscience, Biotechn. Biochem.* 62 (10): 1938-1946