Technical Bulletin

β-Glucosidase Activity Assay Kit

Catalogue number MAK129

Product Description

β-Glucosidase hydrolyzes carbohydrates by acting on terminal, non-reducing β(1→4)-linked D-glucose residues with the release of D-glucose. β-Glucosidases are required by organisms for the consumption of cellulose. Lysozyme, a β-glucosidase present in tears, acts on the β(1→4) glucose bonds present in the peptidoglycan cell wall of Gram-negative bacteria and helps to prevent bacterial infections in the eye. Defects in β-glucosidase activity have been implicated in Gaucher's disease and Parkinson's disease.

The β-Glucosidase Activity Assay kit provides a simple and direct procedure for measuring β-glucosidase activity in biological samples. In this assay, β-glucosidase activity is determined by a reaction in which β-glucosidase hydrolyzes p-nitrophenyl-β-D-glucopyranoside resulting in the formation of a colorimetric (405 nm) product, proportional to the β-glucosidase activity present. One unit of β-Glucosidse is the amount of enzyme that catalyzes the hydrolysis of 1.0 µmole substrate per minute at pH 7.0.

Components

The kit is sufficient for 100 assays in 96 well plates.

Assay Buffer, pH 7.0 Catalog Number MAK129A	24 mL
β-NPG Substrate Catalog Number MAK129B	1.0 mL
Calibrator (equivalent to 250 U/L) Catalog Number MAK129C	10 mL

Reagents and Equipment Required but Not Provided.

- Spectrophotometric multiwell plate reader
- Clear 96 well flat-bottom plate

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This kit is shipped at room temperature and storage at -20 °C, protected from light, is recommended.

Procedure

This assay is based on a kinetic reaction. Use of a multichannel pipette is recommended. Addition of reagents to Samples should be quick and mixing should be brief but thorough. Assays can be executed at either room temperature or 37 °C.

Equilibrate reagents to room temperature before beginning assay.

Sample Preparation

Samples can be prepared in a 50 mM phosphate buffer, pH 7.0, or any other suitable enzyme buffer. The following compounds are known to affect the enzyme activity and should be avoided: thiol (SH)-containing reagents (For example- dithiothreitol, 2-mercaptoethanol, and 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.

Assay Reaction

1. Transfer 20 µL of distilled water to two wells of a clear 96 well plate. Add 200 µL of water into one of these wells and 200 µL of Calibrator to the other well.
2. Prepare the Master Reaction Mix according to the scheme Table 1. The volume shown is enough for one assay well and has a final concentration of 1 mM β-NPG. Prepare enough of the Master Reaction Mix for each Sample well. The Master Reaction Mix should be prepared fresh each time the assay is run.

Table 1.
Master Reaction Mix

Reagent	**Volume**
Assay Buffer	200 µL
β-NPG Substrate	8 µL

3. Transfer 20 µL of each Sample into separate wells of the plate. Transfer 200 µL of the Master Reaction Mix into each of the Sample wells (not the calibrator or water control wells). Tap plate briefly to mix.
4. Measure the initial absorbance at 405 nm $(A_{405})_{initial}$.
5. Incubate the Samples at either room temperature or 37 °C. After 20 minutes, take the final absorbance measurement $(A_{405})_{final}$.

Calculations

β-Glucosidse Activity (units/L)

$$= \frac{(A_{405})_{final} - (A_{405})_{initial}}{(A_{405})_{calibrator} - (A_{405})_{water}} \times 250 \text{ units/L}$$

$(A_{405})_{calibrator}$ = value for calibrator at 20 minutes
$(A_{405})_{water}$ = value for water at 20 minutes

Note: If the $(A_{405})_{final}$ is higher than 1.0, dilute the sample with water and repeat the assay.

One unit of β-Glucosidse is the amount of enzyme that catalyzes the hydrolysis of 1.0 µmole substrate per minute at pH 7.0

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold reagents	Assay Buffer must be at 37 °C or room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in incompatible buffer	Ensure that all buffer reagents are compatible with assay as detailed under sample preparation
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings

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