GLUCOAMYLASE FROM *TRICHODERMA REESEI* EXPRESSED IN *TRICHODERMA REESEI*

New specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI "not specified" was established at the 77th JECFA (2013).

SYNONYMS Amyloglucosidase; γ-amylase; lysosomal α-glucosidase; acid maltase; exo-1,4-α-glucosidase; glucose amylase; γ-1,4-glucan glucohydrolase; acid maltase; and 1,4-α-D-glucan

SOURCES Produced by submerged straight-batch or fed-batch fermentation of a genetically modified non-pathogenic, non-toxigenic strain of *Trichoderma reesei* which contains a gene coding for glucoamylase from *T. reesei.* The enzyme is secreted to the fermentation broth. The cell mass along with the solid waste slurry carrying the residual microorganism is separated from the enzyme by centrifugation and/or filtration. The liquid enzyme filtrate is concentrated by ultrafiltration followed by diafiltration to remove colour. The product is further polish-filtered and formulated using food-grade stabilizing agents and standardized to the desired activity.

Active principles: Glucoamylase

Systematic names and numbers: Glucan 1,4-α-glucosidase; EC 3.2.1.3; CAS No. 9032-08-0

Reactions catalysed: Hydrolysis of terminal (1→4)-linked α-D-glucose residues successively from non-reducing ends of the chains with release of β-D-glucose.

Secondary enzyme activities: No significant levels of secondary enzyme activities

DESCRIPTION Amber liquid

FUNCTIONAL USES Enzyme preparation.
Used in the manufacture of corn sweeteners such as high fructose corn syrup, baking, brewing and potable alcohol manufacture

GENERAL SPECIFICATIONS Must conform to the current edition of the JECFA General Specifications and Considerations for Enzyme Preparations Used in Food Processing.

CHARACTERISTICS

IDENTIFICATION

Glucoamylase activity: The sample shows glucoamylase activity
See description under TESTS.

TESTS

Glucoamylase activity

Principle

Glucoamylase hydrolyses the substrate *p*-nitrophenyl-alpha-D-glucopyranoside (PNPG) to glucose and *p*-nitrophenol (PNP) at alkaline pH. The released PNP is proportional to enzyme activity and measured at 400 nm. Enzyme activity is expressed in GlucoAmylase Units (GAU). One GAU is defined as the amount of glucoamylase that releases one gram of glucose per hour (= 5.6 mmol of glucose per hour) from soluble starch substrate at pH 4.3 and temperature of 30 º.

Apparatus

Spectrophotometer (400 nm)
Water bath with thermostatic control (40 º ± 1)
Water bath with thermostatic control (30 º ± 1)
Vortex mixer
Magnetic stir plate and stir bars
Positive displacement and repeater pipettes

Reagents and solutions

Preparation of Sodium Acetate buffer (0.1 M, pH 4.3):
Weigh 4.4 g of sodium acetate trihydrate ($NaC_2H_3O_2.3H_2O$) and transfer to a 1-litre volumetric flask. Add 800 ml of deionised water and mix until dissolved. Adjust the pH to 4.30 ± 0.05 with glacial acetic acid. Make up the volume to 1000 ml with water and mix. This solution can be stored at 4° for two weeks.

Preparation of Borax Solution (0.1 M, pH 9.2):
Weigh and transfer 19.04 g of sodium borate decahydrate to a 500 ml volumetric flask. Add 400 ml of water and mix until dissolved. Measure pH. Adjust pH, if needed, to 9.20 $\pm$ 0.05. Bring the volume to 500 ml with water and mix. The solution can be stored at room temperature for six weeks.

Preparation of PNPG substrate (1.1 mg/ml) (light sensitive):
Weigh 55.0$\pm$0.5 mg of PNPG substrate (Sigma) in a beaker. Add 40 ml of Sodium Acetate buffer (0.1 M, pH 4.3) and stir on a magnetic stirrer until dissolved (may require heating in a water bath at 30 º). Transfer contents into a 50 ml volumetric flask. Make up the volume to 50 ml. Transfer the solution to an amber bottle for storage. This solution can be stored at 4 º for two weeks.

Preparation of Standard and Sample Solutions:

Preparation of Stock Standard Solution:
Heat a 1 ml aliquot of glucoamylase standard solution with the activity expressed in GAU/ml as reported on the Certificate of Analysis, in a water bath at 40 º for 10 min. Label as Stock Standard Solution.

Preparation of Working Standard Solutions:
Dilute different aliquots of the stock standard with the 0.1M Sodium Acetate Buffer to obtain at least three working standards with concentrations that fall within the linear range of the assay (the linear range of the assay is ~0.5 - 2.4 GAU/ml) after subtracting blank. The

prepared solutions should be kept at room temperature and must be used within 2 h of their preparation.

Preparation of sample:
Heat a 1 ml aliquot of glucoamylase sample in a water bath at 40 º for 10 min. Dilute the glucoamylase sample with the Sodium Acetate buffer. The solution should be kept at room temperature and used within 2 h of its preparation.

Procedure

Preheat water bath to 30 º. Prepare duplicate tubes in a rack and label as Working Standard, Sample and Blank. Using a positive displacement pipette, dispense 250 µl of 0.1M Sodium Acetate buffer, pH 4.3 to each labeled tube.

Using a positive displacement pipette, add 200 µl of each Working Standard and Sample to the respective labeled tubes. Add an additional 200 µl of Sodium Acetate buffer to the blank tube. Place the tubes in the 30 º water bath for 5 min. Add to each set of duplicate tubes, 500 µl of 1.1 mg/ml PNPG substrate solution with a repeater pipette at timed intervals and vortex for 3 sec each. Incubate all tubes in a water bath for 10$\pm$ 0.1 min from the time of addition of the substrate. Remove from the water bath and immediately add 1.0 ml of 0.1M Borax solution at the same time interval used for the substrate addition using a repeater pipette to each tube. Vortex each tube for 3 sec and place on a second rack. Transfer the standards, samples and blanks to 1.5 ml cuvettes. Measure the absorbances at 400 nm, after appropriately zeroing with blank. Prepare the standard curve using linear regression. The correlation coefficient must be ≥0.99. Determine the glucoamylase concentration of each sample from the standard curve.

Weigh sample. Record the value as density in g/ml, up to two significant figures.

Calculate the glucoamylase activity for each sample of the glucoamylase preparation in GAU/g as follows:

$$\text{Glucoamylase activity, GAU/g} = \frac{C \times D}{\text{Sample density}}$$

Where:

C is the glucoamylase concentration of the sample determined from the standard curve (GAU/ml), and

D is the dilution factor of the sample divided by the sample density (g/ml)