MALTOTETRAOHYDROLASE FROM *PSEUDOMONAS STUTZERI* EXPRESSED IN *BACILLUS LICHENIFORMIS*

New specifications prepared at the 80th JECFA (2015) and published in FAO JECFA Monographs 17 (2015). An ADI "not specified" was established at the 80th JECFA (2015).

SYNONYMS Exo-maltotetraohydrolase; 1,4-alpha-D-glucan maltotetraohydrolase

SOURCES Produced by straight-batch or fed-batch fermentation of a genetically modified non-pathogenic, non-toxigenic strain of *Bacillus licheniformis* containing the gene from *Pseudomonas stutzeri*. The enzyme is recovered from the fermentation broth. The recovery process includes the separation of cell mass along with the solid waste slurry carrying the residual microorganism from the enzyme by centrifugation and/or filtration. The liquid enzyme filtrate is concentrated by ultrafiltration followed by polish filtration. The final product is standardized with food-grade materials to the desired activity.

Active principles Maltotetraohydrolase

Systematic names and numbers 4-alpha-D-glucan maltotetraohydrolase; EC 3.2.1.60

Reactions catalyzed Hydrolysis of (1→4)-alpha-D-glucosidic linkages in amylaceous polysaccharides, to remove successive maltotetraose residues from the non-reducing chain ends, using amylaceous polysaccharides like starch as a substrate.

Secondary enzyme activities No significant levels of secondary enzyme activities

DESCRIPTION Amber liquid or off-white granulate

FUNCTIONAL USES Enzyme preparation.
Used in the manufacture of baked goods, and in starch processing.

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

CHARACTERISTICS

IDENTIFICATION

Maltotetraohydrolase activity The sample shows maltotetraohydrolase activity. See description under TESTS.

TESTS

Enzyme Activity

Principle

Maltotetraohydrolase activity is measured by a method using end-blocked *p*-nitrophenyl-maltoheptaoside substrate in the presence of 2 exo-acting enzymes, namely amyloglucosidase and α -glucosidase (maltase). When the oligosaccharide is hydrolyzed by maltotetraohydrolase, the non-blocked nitrophenyl-linked maltooligosaccharide that is produced is hydrolysed to glucose and free *p*-nitrophenol by the combined action of excess amyloglucosidase and α-glucosidase present in the reaction mixture. The amyloglucosidase hydrolyses p-nitrophenyl maltooligosaccharide to glucose and *p*-nitrophenyl α-D-glucoside, and the α -glucosidase hydrolyses the *p*-nitrophenyl α -D-glucoside to glucose and yellow coloured *p*-nitrophenol. The reaction is terminated by the addition of an alkaline solution which assists in optimal colour development.

The *p*-nitrophenol release is proportional to maltotetraohydrolase activity, and is monitored at 410 nm.

One BMU refers to an internal standard with a defined activity of the enzyme. One BMU is defined as the activity degrading 0.0351 mmole per min of blocked *p*-nitrophenyl-α-D-maltoheptaoside in the presence of amyloglucosidase and α-glucosidase at 25°, in a specific assay mix for 5 min.

Apparatus

Spectrophotometer
Water bath with thermostatic control
Positive displacement pipettes

Reagents and solutions

MilliQ water, or equivalent
Blocked *p*-Nitrophenyl-α-d-Maltoheptaoside (BPNPG7), (Sekisui Diagnostics, US# 70-3685-01/International# BLMN-70-3685 or equivalent)
α-Glucosidase (maltase), (Sekisui Diagnostics, US# 70-1235-01/International # MALT-70-1235 or equivalent)
Glucoamylase, (Sekisui Diagnostics: # GLUC-70-6881 or equivalent)
DL-malic acid, (Sigma # M0875 or equivalent)
Sodium Chloride, reagent grade, (Sigma # S9888 or equivalent)
Calcium Chloride, anhydrous, (Sigma # C1016 or equivalent)
Bovine Serum Albumin, (Sigma # A3294 or equivalent)
1N Sodium Hydroxide TS (from J.T. Baker #3728-01 or equivalent)
Boric Acid, (Sigma # B0394 or equivalent)
1,2-Propanediol (Propylene glycol), (Sigma # P4347 or equivalent)
Enzyme standard (standard solution with certified activity expressed in BMU/ml, available from DuPont (Danisco US Inc.), Rochester, NY, USA).

50 mM Malate buffer, pH 5.6 (For Assay buffer)

Dissolve 6.7 g DL-malic acid in 800 ml MilliQ water. Add 2.92 g NaCl and 0.29 g $CaCl_2$ and dissolve. Adjust pH to 5.6 with 1N NaOH. Make up to 1.0 l with MilliQ water.

Assay buffer
1% Bovine Serum Albumin (BSA) in Malate Buffer, pH 5.6:
Weigh an amount of BSA and dissolve in the appropriate volume of 50 mM Malate Buffer, pH 5.6, in order to obtain a 1% (w/v) solution. (Example: 1.0 g BSA dissolved in 100 ml 50 mM Malate Buffer, pH 5.6)

Stop solution (200mM Borate solution)
Dissolve 6.18 g Boric Acid in 400 ml MilliQ water. Adjust pH to 10.2 with 1N NaOH TS. Make up to 500 ml with MilliQ water.

Working substrate solution
Add 54.5 mg BPNPG7, 300 Units α-Glucosidase (maltase), and 120 Units glucoamylase in 10 ml Assay Buffer. Label as Working Substrate Solution.

Standard solutions
Weigh out an amount of enzyme standard, and dilute with Assay buffer to make three dilutions to set up a three point standard curve with a linear range of ~0.2 to 1.6 BMU/ml, at 410 nm. Label accordingly.

Prepare a blank by pipetting 50 µl Assay buffer into a test tube. Label accordingly.
Store at room temperature.

Samples
Liquid sample: Heat liquid samples to 37 - 40° in a water bath for 15 - 30 min to dissolve any precipitate. Dilute the samples with Assay buffer to obtain a final absorbance within the linear range of the assay. Heat the final dilutions of samples to 37 - 40° in a water bath for 15 - 30 min before assaying. Store diluted samples at room temperature.

Granular sample: Weigh out duplicate granular samples (0.5 g-10 g) in a 100 ml beaker. Add approximately 80 ml of assay buffer to each. Stir on a magnetic plate for 20 min. Transfer to a 100 ml volumetric flask, and adjust to volume with Assay buffer. Prepare additional dilutions if necessary, using Assay Buffer, to obtain a final absorbance within the linear range of the standard curve, at 410 nm. Store diluted samples at room temperature.

Procedure
Preheat water bath to 30º.

Prepare tubes, in duplicates, on a rack. Label as Working Standard, Sample and Blank. Using a positive displacement pipette, dispense 50 µl standards and working sample dilutions to each labeled tube. Place the rack in a water bath at 25°. Also place the Working Substrate Solution in the water bath to equilibrate for 5 min.

Using a positive displacement pipette, dispense 400 µl aliquots of Working Substrate Solution into the labelled test tubes, at timed intervals. Incubate all test tubes at 25° for exactly 5 min, in a water bath. Add 600 µl Stop Solution to each test tube and vortex. Transfer the content of each tube to a plastic cuvette. Measure the absorbance at 410 nm, after appropriately zeroing with blank.

Prepare the standard curve using linear regression. The correlation coefficient must be ≥ 0.99. Determine the maltotetraohydrolase

concentration of each enzyme standard and sample from the standard curve.

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

Calculations

Calculate the activity for each sample in BMU/g as follows:

$$\text{Maltotetraohydrolase Activity, BMU per gram} = \frac{\text{C} \times \text{DF}}{\text{W}}$$

Where

C is concentration of maltotetraohydrolase from the standard curve in BMU/ml
DF is Dilution Factor of sample
W is sample density in g/ml