BRANCHING GLYCOSYLTRANSFERASE FROM *RHODOTHERMUS OBAMENSIS* EXPRESSED IN *BACILLUS SUBTILIS*

New specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs7 (2009). An ADI "not specified" was established at the 71st JECFA (2009).

SYNONYMS 1,4-α-glucan branching enzyme; amylo-(1,4→1,6)-transglycosylase; α–glucan-branching glycosyltransferase; branching enzyme; Q-enzyme

SOURCES Branching glycosyltransferase is produced by submerged fed-batch fermentation of a genetically modified strain of *Bacillus subtilis* which contains a gene coding for branching glycosyltransferase from *Rhodothermus obamensis*. The enzyme is recovered from the fermentation broth. The recovery process includes the separation of cellular biomass, concentration by ultrafiltration and/or evaporation, and germ filtration. The final product is formulated using food-grade stabilizing and preserving agents and is standardized to the desired activity.

Active principles: Branching glycosyltransferase

Systematic names and numbers: 1,4-α-D-glucan:1,4-α-D-glucan 6-α-D-(1,4-α-D-glucano)-transferase; EC 2.4.1.18; CAS No. 9001-97-2

Reactions catalysed: Transfers a segment of a 1,4-α-D-glucan chain to a primary hydroxy group in a similar glucan chain to create 1,6-α-linkages

Secondary enzyme activities: No significant levels of secondary enzyme activities

DESCRIPTION Light brown liquid

FUNCTIONAL USES Enzyme preparation.
Used in starch processing to obtain modified starch with improved functional properties

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

CHARACTERISTICS

IDENTIFICATION

<u>Branching glycosyltransferase activity</u>: The sample shows branching glycosyltransferase activity. See description under TESTS.

TESTS

Branching glycosyltransferase activity

Principle

Branching glycosyltransferase catalyses the transfer of a segment of a 1,4-α-D-glucan chain to a primary hydroxy group in a similar glucan chain to create 1,6-α-linkages. The activity of branching glycosyltransferase is determined by measuring the rate of introduction of 1,6-α-linkages into the substrate amylose.

The enzyme activity is expressed in branching enzyme units (BEUs). One BEU is defined as the quantity of the enzyme that causes a decrease in absorbance at 660 nm of an amylose-iodine complex of 1% per minute under standard conditions (pH 7.2; 60°).

Apparatus

Spectrophotometer (660 nm)
Microtitre plates (Nunc F96 MicroWell Plates Cat. No. 467320 (MediSorp) or equivalent)
Water bath with thermostatic control or Eppendorf Thermomixer (60°)
pH meter

Reagents and solutions

0.1 M Tris-HCl buffer (pH 7.2)
(Note: Use distilled water)

S*ubstrate solution:* Accurately weigh (to ± 0.05 mg) 10 mg of amylose type III (Sigma A0512 or equivalent) into a 10-ml volumetric flask. Gently add 0.2 ml of 96% ethanol, make sure that all the amylose is wet, and leave for 3-4 min. Add 0.5 ml of 2 N sodium hydroxide (80 g of NaOH per liter), then 1 ml of water and stir for 10 min to dissolve amylose. Add 0.5 ml of 2 N hydrochloric acid and then 7.7 ml of Tris-HCl buffer. Check the pH of the solution and adjust to 7.2 if necessary. Add Tris-HCl buffer to volume.
The solution should be freshly prepared on the day of use.

Lugols solution: Weigh 0.26 g of iodine and 2.6 g of potassium iodide into a 10-ml volumetric flask. Add water to volume and mix.
The solution should be prepared at least three days before use to ensure that all the iodine has dissolved. The solution is stable for up to 6 months when stored in darkness at room temperature.

Stop reagent: Mix 100 µl Lugols solution, 50 µl of 2 N hydrochloric acid, and 26 ml of water in a measuring cylinder. The reagent should be freshly prepared on the day of use.

Sample solution: Accurately weigh (to ± 0.5 mg) approximately 1 g (W) of the enzyme preparation into a beaker and add about 80 ml of Tris-HCl buffer. Stir the solution slowly for 30 min with the magnetic stirrer to make sure that the sample is completely dissolved. Transfer the solution quantitatively to a 100-ml volumetric flask and add Tris-HCl buffer to volume (V). Dilute

this solution again with Tris-HCl buffer, if necessary, to obtain an activity between 30 and 50 BEU/ml. Calculate the dilution factor (D) and use it in the calculation formula.
(NOTE: Typically, the dilution factor ranges from 10 to 40). The solution should be freshly prepared on the day of use.

Procedure

1. Prepare the following in 2-ml Eppendorf tubes:
   Sample: Mix 50 µl of the sample solution (V_S = 0.050 ml) and 50 µl of the substrate solution (4 replicates)
   Reference: Mix 50 µl of water and 50 µl of the substrate solution (4 replicates)
   Blank: Add 100 µl of water (4 replicates)
2. Mix well and incubate all the tubes at 60° for 30 min (t)
3. After 30 min, add to each tube 2 ml of the stop reagent and mix well
4. Leave for 20 min at room temperature for colour stabilisation
5. Transfer 200 µl of each solution to microtitre plate wells and measure the absorbance at 660 nm
6. Calculate the mean absorbance values based on four replicates for the sample (A_S), reference (A_R), and blank (A_B)

NOTE: The absorbance of the sample should be within 0.15 - 0.3. If the absorbance falls outside this range, dilute the sample again with 0.1 M Tris-HCl (pH 7.2) and repeat the procedure.

Calculation

Use the following formula to calculate the activity of the branching enzyme:

$$\text{Activity (BEU/g)} = \frac{(A_R - A_S) \times V \times D \times 100}{(A_R - A_B) \times t \times V_S \times W}$$

where

$A_R - A_S$ is the difference in absorbance between the reference and sample;
$A_R - A_B$ is the difference in absorbance between the reference and blank;
V is the initial volume of the sample solution (ml);
D is the dilution factor;
100 is the conversion factor to express the enzyme activity in BEU/g;
t is the incubation time (min);
V_S is the volume of the sample solution used in the procedure (ml); and
W is the sample weight (g).