Invertase from Candida sp.

I755075

Invertase from Candida sp. is responsible for catalyzing the hydrolysis of sucrose into glucose and fructose and is widely used in the field of carbohydrate processing. Invertase from Candida sp. can be used for enzymatic determination of sucrose concentration as well as for structural analysis of carbohydrates containing β-D-fructofuranosyl residue.

Preparation and spreparation

Appearance: White amorphous powder, lyophilized

Activity: ≥100U/mg enzyme powder (containing approx. 70 % of stabilizer)

Stabilizer: KH_2PO_4

Storage Conditions

Store at -20℃ long term (12 months). Upon reconstitution, it is recommended to aliquot. Avoid freeze/thaw cycle.

Properties

Stability	Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	approx. 260,000	-
Michaelis constant	1.5×10^{-2} M (Saccharose)	-
Structure	Glycoprotein containing approx. 50% of carbohydrates	-
Optimum pH	3.5−4.0	(Fig.3)
Optimum temperature	60−70 ℃	(Fig.4)
pH Stability	pH 4.0−6.0 (50 ℃, 10min)	(Fig.5)
Thermal stability	below 60 ℃ (pH 4.5, 10min)	(Fig.6)
Substrate specificity	This enzyme hydrolyzes saccharose and raffinose, but does not hydrolyze inulin and melezitose.	-

Applications

This enzyme is useful for enzymatic measurement of saccharose, and structural analysis of carbohydrates containing β-D-fructofuranoside residue.

Assay Principle

$$\text{Saccharose} + H_2O \xrightarrow{\text{invertase}} \text{D-Fructose} + \text{D-Glucose}$$

The formation of reducing sugars is measured by the modified Fehling-Lehmann-Schoorl method.

Unit definition

One unit catalyzes formation of one milligram of glucose-equivalent reducing sugars in 3 minutes, under the conditions detailed below. This activity is equivalent to the international unit, i.e. hydrolysis of one micromole of saccharose per minute at the same temperature.

Method

Reagents

A. Saccharose solution 5.0 %: 5.0 g of saccharose / 100 mL of 80 mM acetate buffer, pH 4.5, with 2−3 drops of toluene added for preservation.

B. Alkaline solution 103 g of NaOH + 346 g of potassium sodium tartrate tetrahydrate / 1,000 mL of H_2O.

C. $CuSO_4$ solution: 6.93% (69.3g $CuSO_4 \cdot 5H_2O$/1,000ml of H_2O).

D. KI solution: 30% (300g KI/1,000ml of H_2O) (Store in a brown bottle).

E. H_2SO_4 solution: 25%.

F. $Na_2S_2O_3$ solution: 50mM (49.638g $Na_2S_2O_3 \cdot 5H_2O$, 4.0g Na_2CO_3 (stabilizer)/4,000ml of H_2O) (Store in a brown bottle and keep for 3−4 days before use).

G. Soluble starch solution: 2.0% (Dissolve by boiling) (Should be prepared fresh).

H. Enzyme diluent: 50mM acetate buffer, pH 4.5.

Procedure

1.Pipette 1.0 mL of substrate solution (A) into a test tube and equilibrate at 20 ℃ for approximately 5 minutes.

2.Add 1.0 mL of the enzyme solution (pre-incubated at 20 ℃) and mix.

Concentration in assay mixture

Acetate buffer	65mM
Saccharose	73mM

3.After exactly 3 minutes at 20 ℃, add 2.0 mL of alkaline solution (B) to stop the reaction. At the same time, prepare the blank by first mixing the substrate solution with 2.0 mL of alkaline solution after incubation for 3 minutes at 20 ℃, then add the enzyme solution, and carry out the same procedure as with the test (Procedure 4−8).

4.Transfer the stopped reaction mixture from the test tube to a 100-mL Erlenmeyer flask. Rinse the inside of the test tube with approximately 3 mL of distilled water and transfer the rinsings to the flask. Repeat this procedure three times.

5.Add 2.0 mL of $CuSO_4$ solution (C) and place the flask directly on an electrothermic heater (1.2 KWH) in the presence of a glass bead (5 mmφ) to prevent bumping.

6.Keep for exactly 2 minutes in a boiling state and cool to room temperature under running water.

7.Add 2.0 mL each of KI solution (D) and H_2SO_4 solution (E) in this order.

8. Shake the flask and determine the amount of residual Cu^{2+} by titration with $Na_2S_2O_3$ solution (F) in the presence of a few drops of soluble starch (G) as an indicator.

9.Record the titers (ml) of the test (t) and the blank (b), and calculate the titration difference

(Δtiter, mL).

*Dissolve the enzyme preparation in ice-cold distilled water and dilute to 2.0−9.0 U/mL with the enzyme dilute (H), immediately before the assay.

Calculation

Activity can be calculated by using the following formulas:

$$\text{Volume activity (U/ml)} = \frac{\Delta\text{titer } (b-t)\times F\times df}{0.600\times Vs} = \Delta\text{titer}\times F\times 1.66\times df$$

Weight activity (U/mg) = (U/ml)×1/C

Vs: Sample volume (1.0mL)

0.600: Titration difference (mL) of 50 mM $Na_2S_2O_3$ solution (F = 1.00) for 1.0 mg of reducing sugar (glucose)

F: Concentration factor of 50 mM $Na_2S_2O_3$ (F should be determined by the iodotimetry method in each time of the preparation).

C: Enzyme concentration in dissolution (c mg/mL)

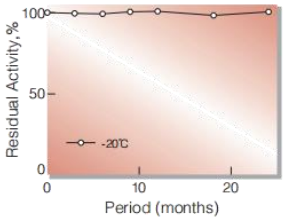

Fig.1. Stability (Powder form)
[kept under dry conditions]

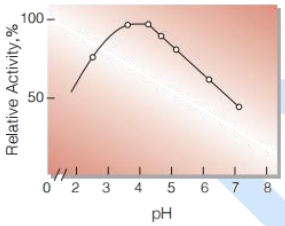

Fig.3. pH-Activity
[20℃, 3min-reaction in the following buffer solution:pH2~3,0.1M glycine-HCl; pH4~5,50mM acetate ; pH6~7,50mM phosphate]

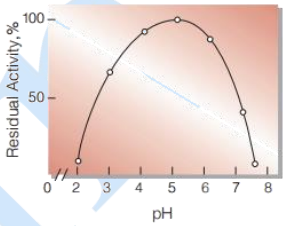

Fig.5. pH-Stability
[50℃, 10min-treatment with the following buffer solution: pH2~3,0.1M glycine-HCl; pH4~5,50mM acetate; pH6~8,50mM phosphate]

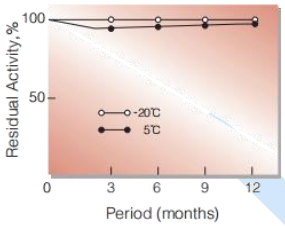

Fig.2. Stability (Powder form)
[kept under dry conditions]

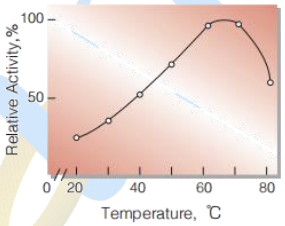

Fig.4. Temperature activity
[3min-reaction in 50mM acetate buffer, pH4.5]

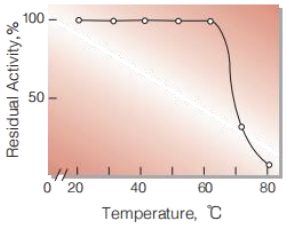

Fig.6. Thermal stability
[10min-treatment with 50mM acetate buffer, pH4.5]