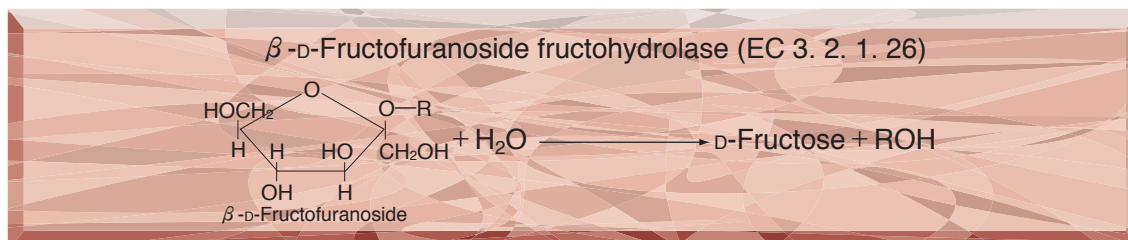


● TOYOBO ENZYMES ●
(Diagnostic Reagent Grade)

INVERTASE

from Candida sp.



PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized
Activity	: Grade I 100U/mg-solid or more (containing approx. 70% of stabilizer)
Stabilizer	: KH ₂ PO ₄

PROPERTIES

Stability	: Stable at -20°C 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°C	(Fig.4)
pH Stability	: pH 4.0-6.0 (50°C, 10min)	(Fig.5)
Thermal stability	: below 60°C (pH 4.5, 10min)	(Fig.6)
Substrate specificity	: This enzyme hydrolyzes saccharose and raffinose, but does not hydrolyze inulin and melezitose. ⁶⁾	

APPLICATIONS

This enzyme is useful for enzymatic determination of saccharose and for the structure investigation of carbohydrates containing β -D-fructofuranoside residue.

ASSAY

Principle:



The appearance of reducing sugars is measured by the modified Fehling-Lehmann-Schoorl method. ⁷⁾

Unit definition:

One unit causes the formation of one milligram of reducing sugars equivalent to the glucose at 3 minutes under the conditions described below (This activity is equivalent to the international unit that hydrolyzes one micromole of saccharose per minute at the same temperature).

Method:

Reagents

- A. Saccharose solution : 5.0% [5.0g saccharose/100ml of 80mM acetate buffer, pH 4.5 (add 2–3 drops of toluene for preservation)]
- B. Alkaline solution : (103g NaOH, 346g Rochelle salt · 4H₂O/1,000ml of H₂O)
- C. CuSO₄ solution : 6.93% (69.3g CuSO₄ · 5H₂O/1,000ml of H₂O)
- D. KI solution : 30% (300g KI/1,000ml of H₂O)(Store in a brownish bottle)
- E. H₂SO₄ solution : 25%
- F. Na₂S₂O₃ solution : 50mM (49.638g Na₂S₂O₃ · 5H₂O, 4.0g Na₂CO₃ (stabilizer)/4,000ml of H₂O)(Store in a brownish 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

- Pipette 1.0ml of substrate solution (A) into a test tube and equilibrate at 20°C for about 5 minutes.
- Add 1.0ml of the enzyme solution (pre-incubated at 20°C) and mix.

Concentration in assay mixture	
Acetate buffer	65mM
Saccharose	73mM

- After exactly 3 minutes at 20°C, add 2.0ml of alkaline solution (B) to stop the reaction.
At the same time, prepare the blank by first mixing the substrate solution with 2.0ml of alkaline solution after a 3 min-incubation at 20°C, followed by the addition of the enzyme solution and carry out the same procedure as the test (Procedure 4–8).
- Transfer the stopped reaction mixture from the test tube to a 100ml volume of Erlenmeyer flask. Rinse the inside of the test tube with about 3ml of distilled water and transfer the rinsings to the flask. Repeat this procedure three times.
- Add 2.0ml of CuSO₄ Solution (C) and place the flask directly on a electrothermic heater (1.2 KWH) in the presence of a glass bead (5mm ϕ) to prevent bumping.
- Keep for exactly 2 minutes in a boiling state and cool down to room temperature under running water.
- Add 2.0ml each of KI solution (D) and H₂SO₄ solution (E) in this order.
- Shake the flask and determine the amount of residual Cu⁺⁺ by titration with Na₂S₂O₃ solution (F) in the presence of a few drops of soluble starch (G) as an indicator.
- Record the titers (ml) of the test (t) and the blank (b), and calculate the titration difference (Δ titer, 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 assay.

Calculation

Activity can be calculated by using the following formula :

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

$$\text{Weight activity (U/mg)} = (\text{U/ml}) \times 1/C$$

V_s : Sample volume (1.0ml)

0.600: Titration difference (ml) of 50mM Na₂S₂O₃ solution (F= 1.00) for 1.0mg of reducing sugar (glucose)

F : Concentration factor of 50mM Na₂S₂O₃ (F should be determined by the iodometry method in each time of the preparation).

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

REFERENCES

- 1) H.Negoro; *J.Agric.Chem.Soc. (Japan)*, **31**, 253 (1957).
- 2) K.Myrbck; *The Enzyme (2nd ed.) Vol. 14*, p.379 (1960), AP.
- 3) T.Kaya; *J.Agr.Chem.Soc. (Japan)*, **38**, 417 (1964).
- 4) J.Hoshino and A.Momose; *J.Gen.Appl.Microbiol.*, **12**, 163 (1966).
- 5) O.Lampen et al.; *J.Biol.Chem.*, **243**, 1573 (1968).
- 6) H.Negoro; *J.Ferment, Technol.*, **51**, 879, (1973).
- 7) T.Yamamoto, J.Kumada and T.Sawai; *Bull.Agric.Biol.Chem.Soc. (Japan)*; **21**, 185 (1957).

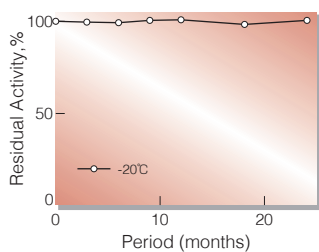


Fig.1. Stability (Powder form)
[kept under dry conditions]

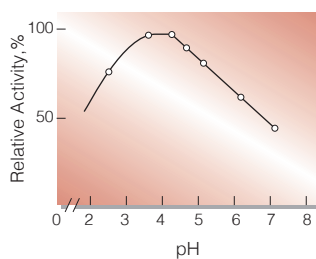


Fig.3. pH-Activity
[20°C, 3min-reaction in the following buffer solution: pH2~3, 0.1M glycine-HCl; pH4~5, 50mM acetate; pH6~7, 50mM phosphate]

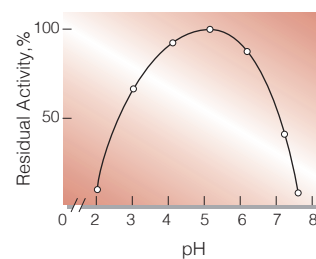


Fig.5. pH-Stability
[50°C, 10min-treatment with the following buffer solution: pH2~3, 0.1M glycine-HCl; pH4~5, 50mM acetate; pH6~8, 50mM phosphate]

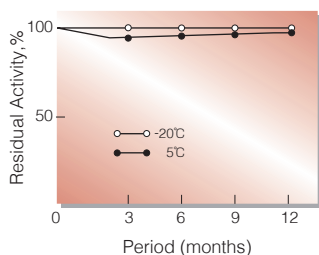


Fig.2. Stability (Powder form)
[kept under dry conditions]

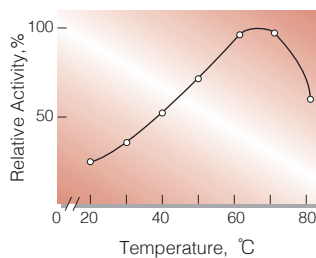


Fig.4. Temperature activity
[3min-reaction in 50mM acetate buffer, pH4.5]

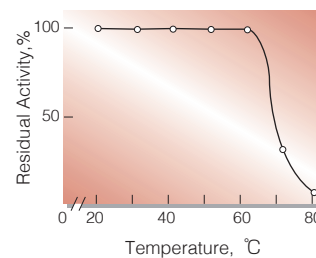


Fig.6. Thermal stability
[10min-treatment with 50mM acetate buffer, pH4.5]

活性測定法 (Japanese)

1.原理

Saccharose + H₂O $\xrightarrow{\text{invertase}}$ D-Fructose + D-Glucose
 還元糖の生成量をFehling-Lehman-Schoorl変法で測定する。

2.定義

下記条件下で3分間に1.0mgのグルコース相当還元糖を生成する酵素量を1単位(U)とする。

3.試薬

- 5.0%サッカロース溶液 [5.0gのサッカロースを80mM酢酸緩衝液, pH4.5に溶解し100mlとする(防腐の為にトルエン2~3滴を滴下しておく)]
- ロッシェル塩アルカリ溶液(103gのNaOH及び346gの酒石酸カリウム・ナトリウム塩・4H₂Oを蒸留水に溶解し, 1,000mlとする)
- 6.93%硫酸銅溶液(69.3gのCuSO₄・5H₂Oを蒸留水に溶解し, 1,000mlとする)
- 30%ヨウ化カリウム溶液(300gのKIを蒸留水に溶解し, 1,000mlとする)(褐色瓶中で保存)
- 25%硫酸溶液
- 50mMチオ硫酸ナトリウム溶液(49.638gのNa₂S₂O₃・5H₂O及び4.0gのNa₂CO₃を蒸留水に溶解し, 4,000mlとする)(褐色瓶中で保存, 調製後3~4日放置して使用する)
- 2.0%可溶性でんぷん溶液(2.0gの可溶性でんぷんを蒸留水100mlに懸濁し煮沸溶解する。指示薬として使用)(用時調製)

酵素溶液：酵素標品を予め氷冷した蒸留水で溶解し、分析直前に50mM酢酸緩衝液, pH 4.5で2.0~9.0 U/mlに希釈する。

4.手順

- 試験管に基質溶液(A) 1.0mlを採り, 20°Cで約5分間予備加温する。
- 予め20°Cに調温されていた酵素溶液1.0mlを加え, 反応を開始する。
- 20°Cで正確に3分間反応させた後, ロッシェル塩アルカリ溶液(B) 2.0ml加えて反応を停止させる。
- 反応停止後の混液を約100ml容三角フラスコへ移し, 更に試験管を約3mlの蒸留水で3回洗浄して三角フラスコへ回収する。
- 硫酸銅溶液(C)を2.0ml加え, 突沸防止の為に直径5mmφのガラス玉1ヶを入れて電気ヒーターで加熱する。
- 沸騰状態で正確に2分間保ったのち, 流水中で室温迄冷却する。
- ヨウ化カリウム溶液(D) 2.0ml及び硫酸溶液(E) 2.0mlをこの順序に加え, よく混和した後50mMチオ硫酸ナトリウム溶液(F)で滴定する。(指示薬として2~3滴の可溶性デンプン液(G)を加えておく)(tml)。
- 盲検は基質溶液(A) 1.0mlを20°Cで3分間放置後, ロッシェル塩アルカリ溶液(B)を加えて混和し, 次いで酵素溶液1.0mlを加えて調製する。以下手順④~⑦を操作して滴定値を求める(b ml)。

5.計算式

$$U/ml = \frac{\text{滴定値の差 (b ml - t ml)} \times F \times \text{希釈倍率}}{0.600 \times 1.0(ml)}$$

$$= \text{滴定値の差} \times F \times 1.66 \times \text{希釈倍率}$$

$$U/mg = U/ml \times 1/C$$

0.600 : 還元糖(グルコース)1.0mgに相当して生ずる50mMチオ硫酸ナトリウム溶液(F=1.00)の滴定値差(ml/mg)

F : 50mMチオ硫酸ナトリウム溶液の濃度補正係数

C : 溶解時の酵素濃度(c mg/ml)