# Sucrose Phosphorylase (SPL-E)

from recombinant *E. coli*

Sucrose : orthophosphate α-D-glucosyltransferase, EC 2.4.1.7

Sucrose + Orthophosphate ⟶ D-Fructose + α-D-Glucose 1-phosphate

## SPECIFICATION

| | | |
|---|---|---|
| Appearance | white lyophilizate | |
| Activity | ≧50 U/mg lyophilizate | |
| Contaminants | NADH oxidase | $\leqq 5.0\times10^{-4}$% |
| | 6-phosphogluconate dehydrogenase | $\leqq 5.0\times10^{-4}$% |
| Stabilizer | sucrose | |
| Storage | at −20°C | |

## PROPERTIES

| | | |
|---|---|---|
| Molecular weight | *ca*. 56 kDa (gel filtration) | |
| Structure | monomer of 56 kDa (SDS-PAGE) | |
| Isoelectric point | 4.6 | |
| Michaelis constants | $3.9\times10^{-2}$ M (sucrose) | |
| | $6.2\times10^{-3}$ M (phosphate) | |
| pH Optimum | 7.5 | (Fig. 1) |
| pH Stability | 5.0–8.0 | (Fig. 2) |
| Optimum temperature | 40°C | (Fig. 3) |
| Thermal stability | below 45°C | (Fig. 4) |
| Stability (liquid form) | stable at 37°C for at least two weeks | (Fig. 5) |
| Stability (powder form) | stable at 30°C for at least two weeks | (Fig. 6) |
| Inhibitors | glucose, glucono-1,5-lactone | |
| Specificity | sucrose (100), maltose (0), starch (0) | |

## ASSAY PROCEDURE

### Principle

$$\text{Sucrose} + \text{Orthophosphate} \xrightarrow{\text{sucrose phosphorylase}} \text{D-Fructose} + \alpha\text{-D-Glucose-1-phosphate}$$

$$\alpha\text{-D-Glucose-1-phosphate} \xrightarrow{\alpha\text{-phosphoglucomutase}} \alpha\text{-D-Glucose-6-phosphate}$$

$$\text{D-Glucose-6-phosphate} + NADP^+ \xrightarrow{\text{glucose-6-phosphate dehydrogenase}} \text{D-Glucono-1,5-lactone-6-phosphate} + NADPH + H^+$$

The appearance of NADPH is measured spectrophotometrically at 340 nm.

### Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 $\mu$mol of NADPH per min at 25°C and pH 6.8 under the conditions described below.

### Reagents

A. Triethanolamine (TEA) buffer, 0.1 M; pH 7.6: dissolve 1.86 g of triethanolamine hydrochloride in 90 ml of distilled water, adjust to pH 7.6 with 5 N NaOH and dilute with distilled water to 100 ml.
B. Potassium phosphate buffer, (a) 0.1 M; pH 6.8, (b) 0.05 M; pH 6.8: (a) mix 0.1 M $KH_2PO_4$ and 0.1 M $K_2HPO_4$ to make a pH 6.8 solution. (b) dilute 0.1 M potassium phosphate buffer (Reagent B (a)) with same volume of distilled water.
C. Sucrose solution, 0.32 M: 11.0 g of sucrose/100 ml of distilled water.
D. EDTA solution, 9.9 mM: 37 mg of EDTA·$Na_2$·$2H_2O$/10 ml of potassium phosphate buffer (Reagent B (b)).
E. $NADP^+$ solution, 12 mM: 9.2 mg of $NADP^+$·Na/1.0 ml of distilled water.
F. D-Glucose-1,6-diphosphate (G-1,6-$P_2$) solution, 0.1 mM: 1.0 mg of G-1,6-$P_2$ cyclohexylammonium salt/10 ml of distilled water.
G. $MgCl_2$ solution, 1.0 M: 4.06 g of $MgCl_2$·$6H_2O$/20 ml of distilled water.
H. $\alpha$-Phosphoglucomutase ($\alpha$-PGM) suspension, 2000 U/ml: crystalline suspension, 10 mg/ml (200 U/mg).
I. Glucose-6-phosphate dehydrogenase (G6PDH) solution: 2000 U/ml of potassium phosphate buffer (Reagent B (b)).

Sample: dissolve the lyophilized enzyme to a volume activity of 0.8–1.5 U/ml in ice-cold TEA buffer (Reagent A) immediately before measurement.

### Procedure

1. Pipette the following reagents into a cuvette (light path: 1 cm).

| | | |
|---|---|---|
| 1.5 ml | Potassium phosphate buffer | (Reagent B (a)) |
| 1.5 ml | Sucrose solution | (Reagent C) |
| 0.03 ml | EDTA solution | (Reagent D) |
| 0.1 ml | $NADP^+$ solution | (Reagent E) |
| 0.1 ml | G-1,6-$P_2$ solution | (Reagent F) |
| 0.05 ml | $MgCl_2$ solution | (Reagent G) |
| 0.01 ml | $\alpha$-PGM suspension | (Reagent H) |
| 0.01 ml | G6PDH solution | (Reagent I) |

2. Equilibrate at 25°C for about 5 min.
3. Add 0.02 ml of sample and mix.
4. Record the increase of absorbance at 340 nm in a spectrophotometer thermostated at 25°C, and calculate the $\Delta A$ per min using the linear portion of the curve ($\Delta A_S$).
   The blank solution is prepared by adding TEA buffer (Reagent A) instead of sample ($\Delta A_0$).

**Calculation**

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_S - \Delta A_0) \times 3.32\,(\text{ml}) \times df}{6.2 \times 0.02\,(\text{ml})} = \Delta A \times 26.8 \times df$$

Weight activity (U/mg) = (U/ml) × 1/$C$

6.2 : Millimolar extinction coefficient of NADPH at 340 nm ($cm^2/\mu mol$)
$df$ : Dilution factor
$C$ : Content of sucrose phosphorylase preparation in sample (mg/ml)

## APPLICATIONS

The enzyme is useful for the determination of inorganic phosphate in clinical analysis.

$$\text{Sucrose} + \text{Orthophosphate} \xrightarrow{\text{sucrose phosphorylase}} \text{D-Fructose} + \alpha\text{-D-Glucose-1-phosphate}$$

$$\alpha\text{-D-Glucose-1-phosphate} \xrightarrow{\alpha\text{-phosphoglucomutase}} \alpha\text{-D-Glucose-6-phosphate}$$

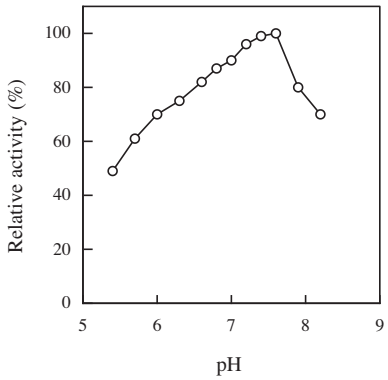
$$\text{D-Glucose-6-phosphate} + NADP^+ \xrightarrow{\text{glucose-6-phosphate dehydrogenase}} \text{D-Glucono-1,5-lactone-6-phosphate} + NADPH + H^+$$

## REFERENCES

Koga, T. *et al*., *Agric. Biol. Chem*., **55**, 1805–1810 (1991).
Kitao, S. and Nakano, E., *J. Ferment. Bioeng*., **73**, 179–184 (1992).

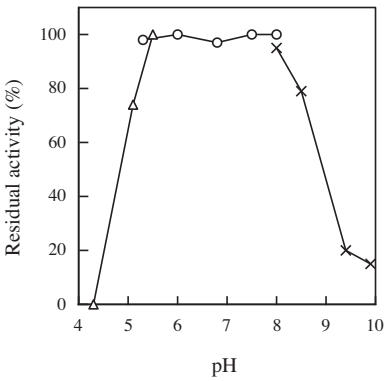
## EXPERIMENTAL DATA

Fig. 1 pH Optimum




Buffer: 50 mM phosphate buffer
(Assay: fructose formed was determined by HPLC)

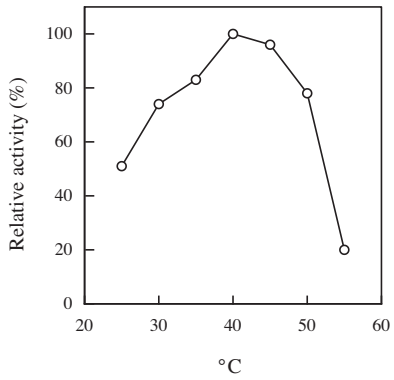
Fig. 2 pH Stability




Treatment: 37°C, 1 h

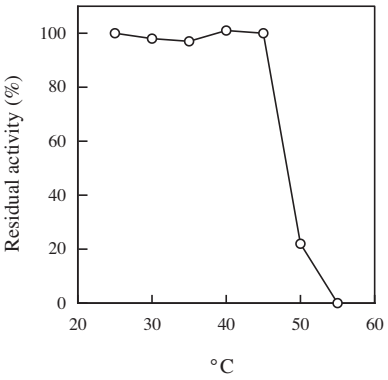
△: 50 mM acetate buffer
○: 50 mM phosphate buffer
×: 50 mM $H_3BO_4$–NaOH buffer

Fig. 3 Optimum temperature



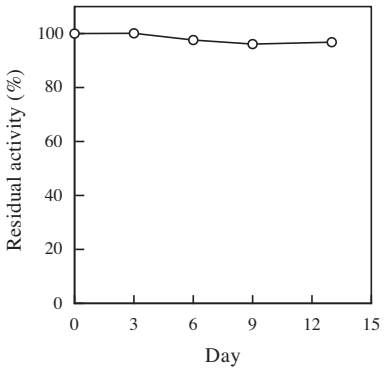

Buffer: 50 mM phosphate buffer, pH 6.8
(Assay: fructose formed was determined by HPLC)

Fig. 4 Thermal stability



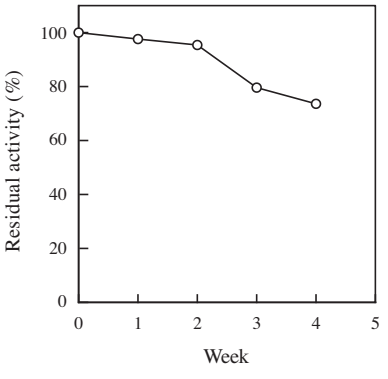

Treatment: 50 mM Tris–HCl buffer, pH 7.5, 10 min

Fig. 5 Stability (liquid form) at 37°C




(Kept in 50 mM phosphate buffer, pH 7.0)

Fig. 6 Stability (powder form) at 30°C




(Kept under dry conditions)