3α-Hydroxysteroid dehydrogenase (3αHSD-EH)

from recombinant *B. choshinensis*

3α-Hydroxysteroid : NAD(P)$^+$ oxidoreductase, EC 1.1.1.50

Androsterone + NAD(P)$^+$ $\longrightarrow$ 5α-Androstane-3,17-dione + NAD(P)H

SPECIFICATION

Appearance	white lyophilizate
Activity	≧30 U/mg
Stabilizer	trehalose
Storage	at −20°C

PROPERTIES

Molecular weight	*ca.* 41 kDa	(gel filtration)
Structure	2 subunits of 25 kDa	(SDS-PAGE)
Michaelis constants	2.4×10^{-5} M	(androsterone)
	3.0×10^{-6} M	(NAD)
pH Optimum	11.0	(Fig. 1)
pH Stability	6.0–10.0	(Fig. 2)
Optimum temperature	50–60°C	(Fig. 3)
Thermal stability	below 45°C	(Fig. 4)
Stability (powder form)	stable at 37°C for at least four weeks	(Fig. 5)

ASSAY PROCEDURE

Principle

The appearance of NADH is measured spectrophotometrically at 340 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 μmol of NADH per min at 25°C and pH 8.9 under the conditions described below.

Reagents

A. Sodium pyrophosphate buffer, 0.1 M; pH 8.9: mix 0.1 M sodium pyrophosphate solution and 0.1 M pyrophosphoric acid solution to make a pH 8.9 solution.
B. Androsterone solution, 4 mM: 58 mg of androsterone/50 ml of methanol.
C. NAD^+ solution, 15 mM; pH 7.0–7.5: dissolve 319 mg of NAD^+ in 25 ml of distilled water, adjust to pH 7.0–7.5 with solid $NaHCO_3$ and dilute with distilled water to 30 ml.
D. Enzyme dilution buffer: mix 30 mM KH_2PO_4 solution and 30 mM K_2HPO_4 solution to make a pH 7.2 solution and add bovine serum albumin (0.1 g/100 ml of the buffer).

Sample: dissolve the enzyme preparation to a volume activity of 0.1–0.4 U/ml in ice-cold enzyme dilution buffer (Reagent D) immediately before measurement.

Procedure

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

1.0 ml	Sodium pyrophosphate buffer	(Reagent A)
0.2 ml	NAD^+ solution	(Reagent C)
1.6 ml	Distilled water	

2. Equilibrate at 25°C for about 5 min.
3. Add 0.1 ml of sample and 0.1 ml of androsterone solution (Reagent B) and mix.
4. Record the increase of absorbance at 340 nm in a spectrophotometer thermostated at 25°C, and calculate the ΔA per min using the linear portion of the curve (ΔA_S).
   The blank solution is prepared by adding enzyme dilution buffer (Reagent D) instead of sample (ΔA_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_S - \Delta A_0) \times 3.0\,(\text{ml}) \times df}{6.2 \times 0.1\,(\text{ml})} = \Delta A \times 4.84 \times df$$

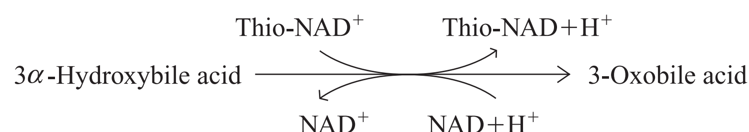
Weight activity (U/mg) = (U/ml) × 1/*C*

6.2 : Millimolar extinction coefficient of NADH ($cm^2/\mu mol$)
df : Dilution factor
C : Content of 3α-hydroxysteroid dehydrogenase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of bile acids in clinical analysis.

Enzyme cycling method:



Colorimetric method:

REFERENCES

Talalay, P., "The Enzymes," Vol. 7 (2nd ed.), Academic Press, New York and London, 1963, pp. 177–202.
Palmer, R., "Methods in Enzymology," Vol. 15, Academic Press, New York and London, 1969, pp. 280–288.
Möbus, E. and Maser, E., *J. Biol. Chem.,* **273**, 30888–30896 (1998).

EXPERIMENTAL DATA

Fig. 1 pH Optimum

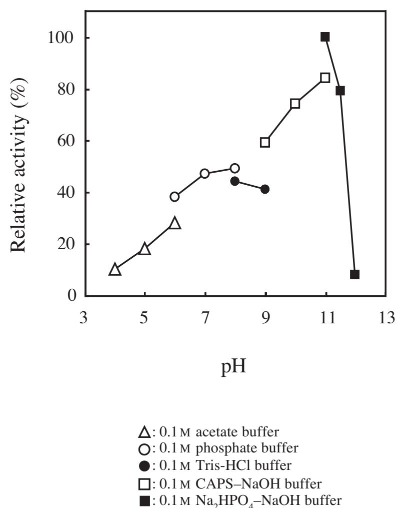


Fig. 2 pH Stability

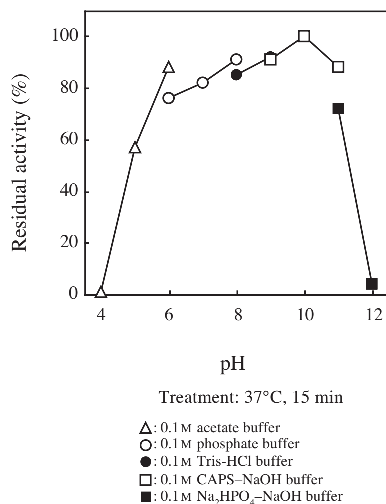
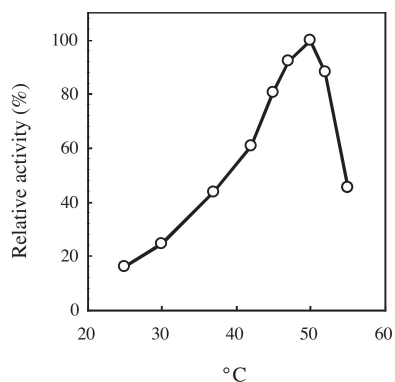
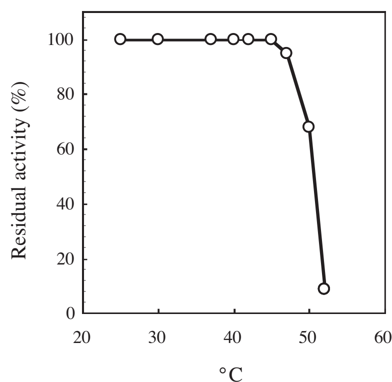


Fig. 3 Optimum temperature



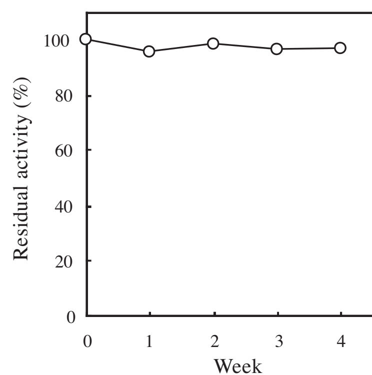
Buffer: 0.1 M sodium pyrophosphate buffer, pH 8.9

Fig. 4 Thermal stability



Treatment: 0.1 M sodium pyrophosphate buffer, pH 8.9, 15 min

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



Buffer: 0.1 M sodium pyrophosphate buffer, pH 8.9