●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

FORMALDEHYDE DEHYDROGENASE

from Pseudomonas sp.

Formaldehyde : NAD^+ oxidoreductase (EC 1.2.1.46)

$HCHO + NAD^+ + H_2O \longrightarrow HCOOH + NADH + H^+$

(Glutathione is not required the catalytic reaction)

PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized
Activity	: Grade Ⅱ 1.0 U/mg-solid or more (containing approx. 70% of stabilizers)
Contaminant	: NADH oxidase $\leq 1.0 \times 10^{-1}$%
Stabilizers	: Mg^{++}, Ca^{++}, bovine serum albumin, glycine, lysine

PROPERTIES

Stability	: Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	: approx. 150,000 (by gel filtration)	
Isoelectric point	: 5.25 [1)]	
Michaelis constants	: 8.0×10^{-5}M (HCHO), 1.2×10^{-4}M (NAD^+)	
Structure	: 2 subunits (75,000) per mol of enzyme[1)]	
Inhibitors	: Chelating agents, Ni^{++}, Cd^{++}, Hg^{++}, PCMB, ionic detergents	
Optimum pH	: 9.0	(Fig.3)
Optimum temperature	: 40℃	(Fig.4)
pH Stability	: pH 8.0−10.0 (30℃, 16hr)	(Fig.5)
Thermal stability	: below 40℃ (pH 7.5, 30min)	(Fig.6)
Substrate specificity	: (Table 1)	
Effect of various chemicals	: (Table 2,3)	

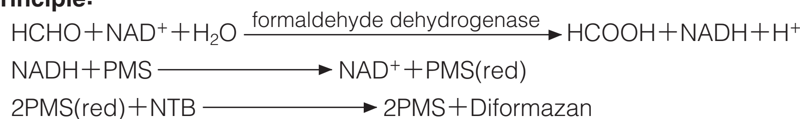
APPLICATIONS

This enzyme is useful for enzymatic determination of formaldehyde and of hydrogen peroxide when coupled with catalase.

ENZYMES Diagnostic Reagent Grade

ASSAY

Principle:



The appearance of diformazan formed by the reduction of nitrotetrazorium blue (NTB) with phenazine methosulfate (PMS)(red) is measured at 570nm by spectrophotometry.

Unit definition:

One unit causes the formation of one half micromole of diformazan per minute under the conditions described below.

Method:

Reagents

- A. HCHO solution : 10mM [0.08ml of 37% (W/V) HCHO/100ml of 50mM phosphate buffer, pH 7.5 contg. 0.5% Triton X-100] (Should be prepared fresh)
- B. NAD^+ solution : 0.4% (40mg $NAD^+ \cdot 3H_2O$/10ml of H_2O)(Should be prepared fresh)
- C. PMS-NTB solution : 1.0mg phenazine methosulfate (PMS), 10mg nitrotetrazorium blue (NTB)/10ml of H_2O (Store at 4℃ in brownish bottle)
- D. HCl solution : 0.3N
- E. Enzyme diluent : 50mM phosphate buffer, pH 7.5 contg. 0.2% BSA

Procedure

1. Prepare the following reaction mixture in a test tube and equilibrate at 37℃ for about 5 minutes

0.5ml	Substrate solution	(A)
0.1ml	NAD^+ solution	(B)
0.1ml	PMS-NTB solution	(C)

Concentration in assay mixture	
Phosphate buffer	21 mM
HCHO	4.2 mM
NAD	0.46mM
PMS	27 μM
NTB	0.10mM
Triton X-100	0.21 %

2. Add 0.5ml of the enzyme solution* and mix.
3. After exactly 15 minutes at 37℃, add 3.0ml of HCl solution (D) to stop the reaction and measure the optical density at 570nm against water (OD test).

   At the same time, prepare the blank by first mixing the reaction mixture with 3.0ml of HCl solution after 15 min-incubation at 37℃, followed by the addition of the enzyme solution (OD blank).

* Dissolve the enzyme preparation in ice-cold enzyme diluent (E) and dilute to 0.01−0.02U/ml with the same buffer, immediately before assay.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta OD\ (OD\ test - OD\ blank) \times Vt \times df}{20.1 \times 1.0 \times t \times Vs} = \Delta OD \times 0.0279 \times df$$

Weight activity (U/mg)=(U/ml)×1/C

- Vt : Total volume (4.2ml)
- Vs : Sample volume (0.5ml)
- 20.1 : Half a millimolar extinction coefficient of diformazan (㎠/0.5micromole)
- 1.0 : Light path length (cm)
- t : Reaction time (15 minutes)
- df : Dilution factor
- C : Enzyme concentration in dissolution (c mg/ml)

REFERENCES

1) M.Ando, T.Yoshimoto, S.Ogushi, K.Rikitake, S.Shibata and D.Tsuru; ***J.Biochem., 85,*** 1165 (1979).

2) Application

D.Tsuru; Creatinine.***Rinsho Kensa (Supple), 22,*** 1331 (1978).

Table 1. Substrate Specificity of Formaldehyde dehydrogenase

Substrate(final 50mM)	Relative activity	Substrate(final 50mM)	Relative activity
HCHO	100	CH_3OH	0
CH_3CHO	47	CH_3CH_2OH	0
$CH_3 \cdot CH_2CHO$	5	$CH_2OH \cdot CH_2OH$	0
$(CH_3)_2CH \cdot CHO$	0	$CH_2(OH) \cdot CH(OH)CH_2OH$	0
$CH_3(CH_2)_2CHO$	3	HCOOH	0
CHO • CHO	19	CH_3CH_2COOH	0
$CH_3 \cdot CO \cdot CHO$	34	COOH • COOH	0

Table 2. Effect of Various Chemicals on Formaldehyde dehydrogenase
(Residual activity after 30 min-treatment at 30℃)

Chemical	Concn.(mM)	Residual activity	Chemical	Concn.	Residual activity
$NiCl_2$	1.0	3%	$MgCl_2$	1.0	124
$Zn(OAc)_2$	1.0	31	$Pb(OAc)_2$	1.0	103
$MnCl_2$	1.0	29	$HgCl_2$	1.0	0.4
$CoSO_4$	1.0	96	$Cd(OAc)_2$	1.0	1.0
$FeCl_3$	1.0	95	EDTA	10	53
$FeSO_4$	1.0	89	NaCl	50	100
$BaCl_2$	1.0	110	KBr	50	93
$Ca(OAc)_2$	1.0	122	KI	50	51

Ac, CH_3CO; EDTA, Ethylenediaminetetraacetate

Table 3. Effect of Various Detergents on Formaldehyde dehydrogenase
(Residual activity after 30 min-treatment at 30℃)

Detergent	Concn.	Residual activity	Detergent	Concn.	Residual activity
Emulgen	0.1	101	Triton X-100	0.1%	105
Brij 35	0.1	101	Na-cholate	0.1	100
Brij 58	0.1	102	DAC*	0.1	9
Span 20	0.1	102	SDS**	10 mM	38
Span 80	0.1	99	NLS***	10 mM	76
Tween 20	0.1	100			

* Dimethyl-benzyl-alkyl-ammonium chloride
** Sodium dodecyl sulfate
*** N-Lauroyl sarcosine-Na salt

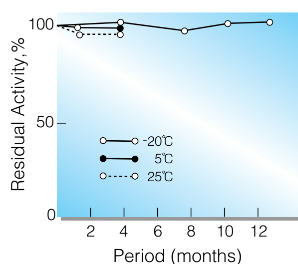


Fig.1. Stability (Powder form)
〔kept under dry conditions〕

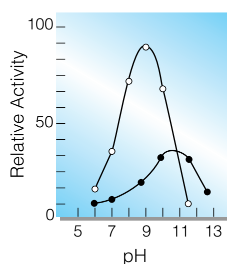


Fig.3. pH-Activity
〔37℃,15min-reaction in 50mM buffer solution:pH6-7.6,phosphate; pH7.2-8.8, Tris-HCl;pH8.7-11 Na_2CO_3;pH11.5-12.5;NaOH-KCl ○,activity for formaldehyde; ●,activity for n-butanol〕

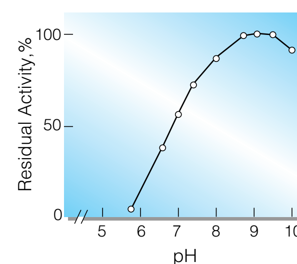


Fig.5. pH-Stability
〔30℃,16hr-treatment with 50mM buffer solution: pH5.8, acetate;pH6.8-8.0, phosphate pH8.0-10.0 borate〕

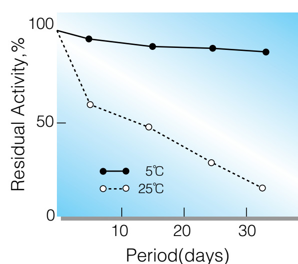


Fig.2. Stability (Liquid form)
〔enzyme concetration: 10U/ml buffer composition:50mM K-phosphate buffer, pH7.5〕

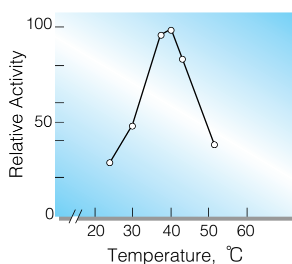


Fig.4. Temperature activity
〔15min-reaction in 50mM phosphate buffer, pH7.5〕

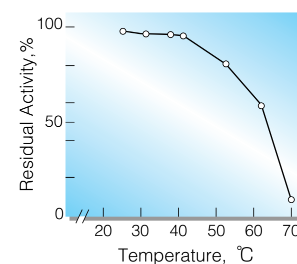
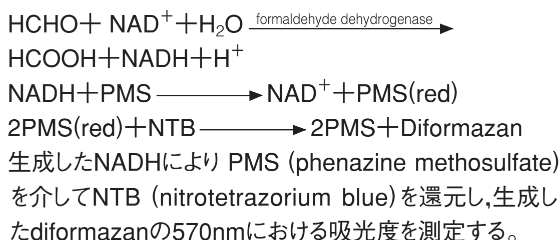


Fig.6. Thermal stability
〔30min-treatment with 50mM phosphate buffer,pH7.5〕

活性測定法（Japanese）

1.原理



$HCHO + NAD^+ + H_2O \xrightarrow{\text{formaldehyde dehydrogenase}} HCOOH + NADH + H^+$

$NADH + PMS \longrightarrow NAD^+ + PMS(red)$

$2PMS(red) + NTB \longrightarrow 2PMS + Diformazan$

生成したNADHにより PMS (phenazine methosulfate)を介してNTB (nitrotetrazorium blue)を還元し,生成したdiformazanの570nmにおける吸光度を測定する。

2.定義

下記条件下で1分間あたり1/2マイクロモルのdiformazanを生成する酵素量を1単位 (U)とする。

3.試薬

A. 10mM HCHO溶液〔0.08㎖の37%(W/V)HCHO溶液を0.5%トリトンX-100を含む50mMリン酸緩衝液, pH7.5で100㎖とする〕(用時調製)

B. 0.4%NAD^+水溶液(40㎎のNAD・$3H_2O$を10㎖の蒸留水に溶解する)(用時調製)

C. PMS-NTB水溶液(1.0㎎PMS及び10㎎NTBを10㎖の蒸留水に溶解する)(褐色瓶中で4℃保存)

D. 0.3N HCl溶液

酵素溶液：酵素標品を予め氷冷した0.2%BSAを含む50mMリン酸緩衝液,pH7.5で溶解し,分析直前に同緩衝液で0.01～0.02U/㎖に希釈する。

4.手順

①試験管に下記反応混液を調製し,37℃で約5分間予備加温する。

0.5㎖	基質溶液	(A)
0.1㎖	NAD^+溶液	(B)
0.1㎖	PMS-NTB水溶液	(C)

②酵素溶液0.5㎖を加え,反応を開始する。

③37℃で正確に15分間反応させた後,HCl溶液(D)3.0㎖を加えて反応を停止させる。この液につき570nmにおける吸光度を測定する(ODtest)。

④盲検は反応混液①を37℃で15分間放置後,HCl溶液(D)3.0㎖を加えて混和し,次いで酵素溶液0.5㎖を加えて調製する。以下同様に吸光度を測定する(ODblank)。

5.計算式

$$U/㎖ = \frac{\Delta OD\ (OD\ test - OD\ blank) \times 4.2(㎖) \times 希釈倍率}{20.1 \times 1.0 \times 15(分) \times 0.5(㎖)}$$

$$= \Delta OD \times 0.0279 \times 希釈倍率$$

U/mg ＝ U/㎖×1 / C

20.1 ： diformazanの1/2ミリモル分子吸光係数(㎠/0.5micromole)

1.0 ： 光路長(cm)

C ： 溶解時の酵素濃度(c mg/㎖)