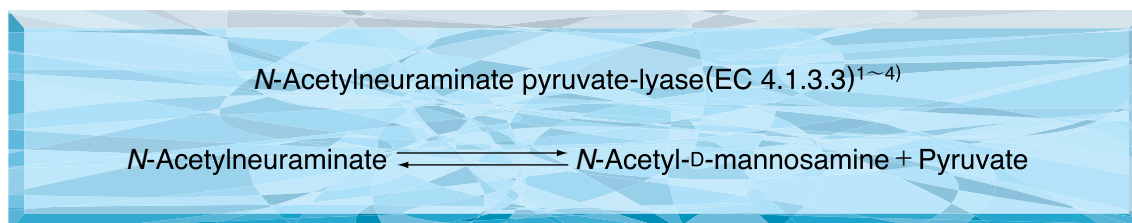


● TOYOBO ENZYMES ●  
(Diagnostic Reagent Grade)

# N-ACETYLNEURAMINIC ACID ALDOLASE

*from Microorganism*



## PREPARATION and SPECIFICATION

Appearance	: Yellowish amorphous powder, lyophilized
Activity	: Grade III 15U/mg-solid or more (30U/mg-protein or more) (containing approx. 30% of stabilizers)
Contaminants	: Catalase $\leq 1.0\%$ NADH oxidase $\leq 1.0 \times 10^{-3}\%$
Stabilizers	: Mannitol, EDTA

## PROPERTIES

Stability	: Stable at $-20^{\circ}\text{C}$ for at least 6 months	(Fig.1)
Molecular weight	: approx. 98,000	
Isoelectric point	: $4.6 \pm 0.1$	
Michaelis constant	: $2.5 \times 10^{-3}\text{M}$ (N-Acetylneuraminic acid)	
Structure	: 3 subunits (approx. 35,000) per mol of enzyme	
Inhibitors	: p-Chloromercuribenzoate, sodium dodecyl sulfate, $\text{Hg}^{++}$ , $\text{Ag}^{+}$	
Optimum pH	: 7.5–8.0	(Fig.2)
Optimum temperature	: $70^{\circ}\text{C}$	(Fig.3)
pH Stability	: pH 6.0–9.0 ( $10^{\circ}\text{C}$ , 25hr)	(Fig.4)
Thermal stability	: below $65^{\circ}\text{C}$ (pH 7.5, 30min)	(Fig.5)
Effect of various chemicals	: (Table 1)	

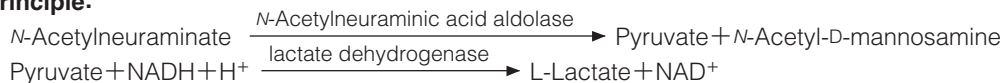
## APPLICATIONS

This enzyme is useful for enzymatic determination of N-acetylneuraminic acid and sialic acid when coupled with the related enzymes in clinical analysis. <sup>5~7)</sup>

For industrial use, this enzyme is useful for enzymatic synthesis of sialic acid. <sup>8~9)</sup>

## ASSAY

### Principle:



The disappearance of NADH is measured at 340 nm by spectrophotometry.

### Unit definition:

One unit causes the oxidation of one micromole of NADH per minute under the conditions described below.

### Method:

#### Reagents

- A. NANA solution : 50mM [Dissolve 309mg of *N*-acetylneuraminic acid (MW=309) in ca. 15ml of 50mM K-phosphate buffer, pH 7.5 and, after adjusting the pH to 7.5 with 1N KOH, fill up to 20ml with the same buffer.] (Stable for at least one week if stored at 0–5°C)
- B. LDH solution : ca. 50U/ml [Dilute pig heart lactate dehydrogenase (Toyobo Grade II, ammonium sulfate suspension) to a concentration of ca. 50U/ml with ice-cold 50mM K-phosphate buffer, pH 7.5] (Should be freshly prepared)
- C. NADH solution : 1.0mM [Dissolve 7.6mg of NADH · Na<sub>2</sub> · 3H<sub>2</sub>O (MW=763) in 10ml of 50mM K-phosphate buffer, pH 7.5] (Should be freshly prepared)
- D. Buffer solution : 50mM K-phosphate buffer, pH 7.5
- E. Enzyme diluent : 50mM K-phosphate buffer, pH 7.5 containing 0.2% BSA

### Procedure

1. Prepare the following reaction mixture in a cuvette (d = 1.0cm) and equilibrate at 37°C for about 5 minutes.

1.0ml	Substrate solution	(A)
0.5ml	LDH solution	(B)
0.5ml	NADH solution	(C)
0.4ml	Buffer solution	(D)

Concentration in assay mixture	
K-Phosphate buffer	50 mM
NANA	20 mM
NADH	0.2mM
LDH	ca.10 U/ml

2. Add 0.1ml of the enzyme solution\* and mix by gentle inversion.
3. Record the decrease in optical density at 340nm against water for 3 to 4 minutes in spectrophotometer thermostated at 37°C, and calculate the  $\Delta OD$  per minute from the initial linear portion of the curve ( $\Delta OD$  test).

At the same time, measure the blank rate ( $\Delta OD$  blank) using the same method the test except that the enzyme diluent (E) is added instead of the enzyme solution.

- \* Dissolve the enzyme preparation in ice-cold enzyme diluent (E) and dilute to 0.1–0.3U/ml with the same buffer, immediately before assay.

### Calculation

Activity can be calculated by using the following formula :

$$\begin{aligned}
 \text{Volume activity (U/ml)} &= \frac{\Delta OD/\text{min} (\Delta OD \text{ test} - \Delta OD \text{ blank}) \times V_t \times df}{6.22 \times 1.0 \times V_s} \\
 &= \Delta OD/\text{min} \times 4.02 \times df
 \end{aligned}$$

Weight activity (U/mg) = (U/ml) × 1/C

V<sub>t</sub> : Total volume (2.5ml)

V<sub>s</sub> : Sample volume (0.1ml)

6.22 : Millimolar extinction coefficient of NADH (cm<sup>2</sup>/micromole)

1.0 : Light path length (cm)

df : Dilution factor

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

## REFERENCES

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- 3) S.B.Arden, W.Chang and L.Barksdale; *J.Bacteriol.*, **112**, 1260 (1972).
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- 6) K.Taniuchi, Y.Miyamoto, Y.Uchida, K.Chifu, M.Mukai, N.Yamaguchi, Y.Tsukada, T.Sugimori, K.Doi and S.Baba; *J.Med.Technol. (Japanese)*, **7**, 403 (1979).
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- 9) Ethan S.Simon, Mark D.Bednarski, and George M.Whitesides; *J.Am.Chem.Soc.*, **110**, 7159 (1988).

Table 1. Effect of Various Chemicals on *N*-Acetylneuraminic acid aldolase

[The enzyme dissolved in 0.1M Tris-HCl buffer, pH7.5 (5U/ml) was incubated at 30°C for 1hr.]

Chemical	Concn.(mM)	Residual activity	Chemical	Concn.(mM)	Residual activity
None	—	100%	PCMB	2.0	0%
Metal salt	2.0		NEM	2.0	103
MgCl <sub>2</sub>		107	NaF	2.0	100
CaCl <sub>2</sub>		87	NaN <sub>3</sub>	20	100
Ba(OAc) <sub>2</sub>		95	EDTA	5.0	95
FeCl <sub>3</sub>		89	o-Phenanthroline	2.0	100
CoCl <sub>2</sub>		93	$\alpha, \alpha'$ -Dipyridyl	2.0	101
MnCl <sub>2</sub>		98	Borate	50	86
ZnSO <sub>4</sub>		92	Triton X-100	0.10%	109
NiCl <sub>2</sub>		99	Na-cholate	0.10%	95
CuSO <sub>4</sub>		64	SDS	0.10%	0
Pb(OAc) <sub>2</sub>		87	Tween 40	0.10%	96
AgNO <sub>3</sub>		0	Span 85	0.10%	93
HgCl <sub>2</sub>		0			

Ac, CH<sub>3</sub>CO; PCMB, p-Chloromercuribenzoate; NEM, N-Ethylmaleimide, EDTA, Ethylenediaminetetraacetate; SDS, Sodium dodecyl sulfate.

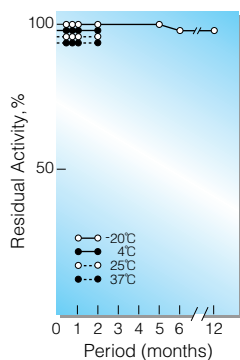


Fig.1. Stability (Powder form)

(kept under dry conditions)

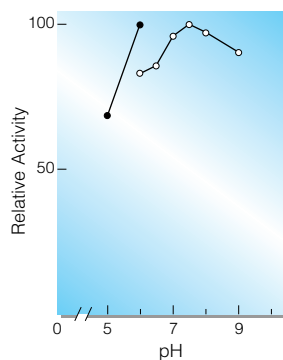


Fig.2. pH-Activity

[37°C, 5min-reaction in 50mM buffer solution : pH5.0-6.0, acetate; pH6.0-9.0, K-phosphate; The enzyme activity was assayed by the 2,4-dinitrophenyl-hydradine method.]

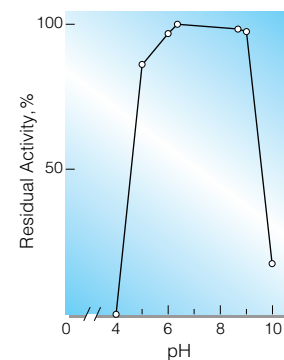


Fig.4. pH-Stability

[10°C, 25hr-treatment with 50mM buffer solution : pH4.0-6.0, acetate; pH6.0-9.0, K-phosphate ; pH9.0-10.0, borate.]

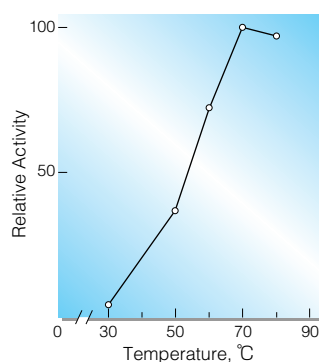


Fig.3. Temperature activity

[5min-reaction in 50mM K-phosphate buffer pH7.5. The enzyme activity was assayed by the 2,4-dinitrophenyl-hydradine method.]

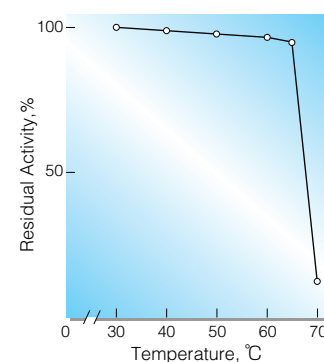
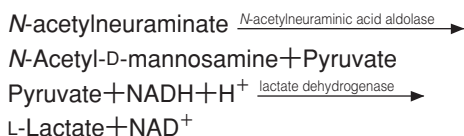


Fig.5. Thermal stability

[30min-treatment with 50mM K-phosphate buffer, pH7.5, enzyme concentration.: 20U/ml]

## 活性測定法 (Japanese)

### 1.原理



NADHの消失量を340nmの吸光度の変化で測定する。

### 2.定義

下記条件で1分間に1マイクロモルのNADHが酸化される酵素量を1単位(U)とする。

### 3.試薬

- A. 50mM NANA溶液 [309mgのN-アセチルノイラミン酸(MW=309)を約15mlの50mM K-リン酸緩衝液, pH7.5に溶解し, 1N KOHでpHを7.5に調整後, 同緩衝液で20mlとする] (0~5℃保存で1週間は使用可能)
  - B. LDH溶液 [ブタ心臓LDH(東洋紡製Grade II, 硫酸懸濁液)を50mM K-リン酸緩衝液, pH7.5で, 約50U/mlに希釈する] (用時調製)
  - C. 1.0mM NADH溶液 [7.6mgのNADH・Na<sub>2</sub>・3H<sub>2</sub>O (MW=763)を10mlの50mM K-リン酸緩衝液, pH7.5に溶解する] (用時調製)
  - D. 50mM K-リン酸緩衝液, pH7.5
- 酵素溶液: 酵素標品を予め氷冷した0.2%のBSAを含む50mM K-リン酸緩衝液, pH7.5 (D)で溶解し, 分析直前に同緩衝液で0.1~0.3U/mlに希釈する。

### 4.手順

- ①下記反応混液をキュベット(d=1.0cm)に調製し, 37℃で約5分間予備加温する。
 

1.0ml	基質溶液	(A)
0.5ml	LDH溶液	(B)
0.5ml	NADH溶液	(C)
0.4ml	K-リン酸緩衝液	(D)
- ②酵素溶液0.1mlを添加し, ゆるやかに混和後, 水を対照に37℃に制御された分光光度計で340nmの吸光度変化を3~4分間記録し, その初期直線部分から1分間当りの吸光度変化を求める(ΔOD test)。
- ③盲検は反応混液①に酵素溶液の代りに酵素希釈液(0.2%BSAを含む50mM K-リン酸緩衝液, pH7.5)0.1mlを加え, 上記同様に操作を行って, 1分間当りの吸光度変化を求める(ΔOD blank)。

### 5.計算式

$$\begin{aligned}
 \text{U/ml} &= \frac{\Delta \text{OD}/\text{min} (\Delta \text{OD test} - \Delta \text{OD blank}) \times 2.5(\text{ml}) \times \text{希釈倍率}}{6.22 \times 1.0 \times 0.1(\text{ml})} \\
 &= \Delta \text{OD}/\text{min} \times 4.02 \times \text{希釈倍率} \\
 \text{U/mg} &= \text{U/ml} \times 1 / C \\
 6.22 &: \text{NADHのミリモル分子吸光係数} \\
 &(\text{cm}^2/\text{micromole}) \\
 1.0 &: \text{光路長(cm)} \\
 C &: \text{溶解時の酵素濃度(c mg/ml)}
 \end{aligned}$$