

(Diagnostic Reagent Grade)

T-67

NAD SYNTHETASE [NADS II]

from *Geobacillus stearothermophilus*
(Deamido-NAD⁺ : ammonia ligase (AMP-forming), EC 6.3.1.5)
(NAD synthase)



Preparation and Specification

Appearance : White amorphous powder, lyophilized
Specific activity : More than 1.00 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 50 kDa (gel filtration) 25 kDa (SDS-PAGE)	
Michaelis constants	: Deamido-NAD $2.4 \times 10^{-5}\text{M}$ ATP $4.3 \times 10^{-5}\text{M}$ NH ₃ $2.16 \times 10^{-3}\text{M}$	
Isoelectric point	: pH 5.2 ± 0.2	
Optimum pH	: 9.0–10.5	Figure 1
pH stability	: 6.0–9.0 (37°C, 15 min)	Figure 2
Optimum temperature	: 70°C (Tris-HCl buffer)	Figure 3
Thermal stability	: Stable at 60°C and below (pH 7.5, 10 min)	Figure 4
Effect of metal ions	: See Table 2	
Effect of detergents	: See Table 3	

Applications for diagnostic Test

This enzyme is useful for enzymatic determination of **ATP, ammonia, urea or creatinine** when coupled with creatinine deiminase.

This enzyme is suitable for **enzymatic cycling method** when coupled with dehydrogenase and diaphorase (T-06).

Please refer to an information 3α-hydroxysteroid dehydrogenase (T-58).

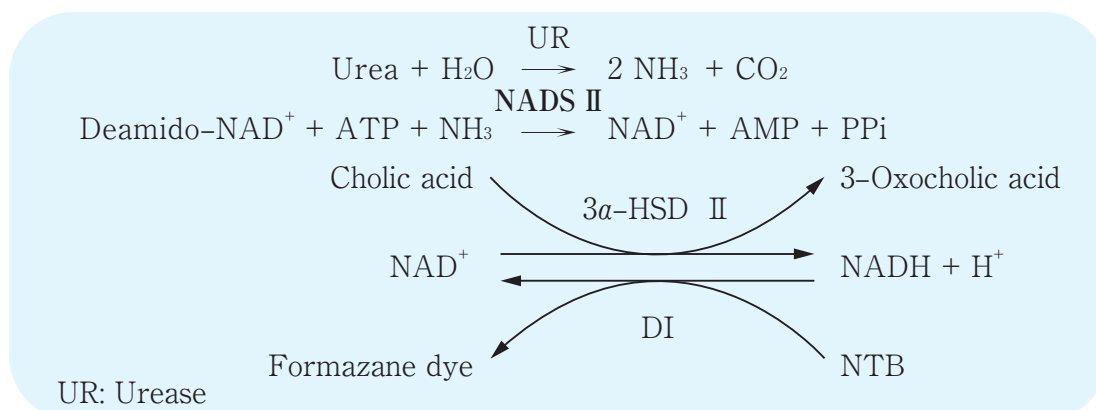


Table 1. Substrate specificity

Substrate (1mM)	Relative activity (%)
NH ₄ Cl	100
L-aminoacids (Leu, Arg, Lys, Ileu, Tyr, Ala, Glu, Gln, Asn, Gly, Ser)	0
Azaserine	0
Urea	0
Uric acid	0
Creatinine	0
Creatine	0
Tris	0
Good's buffers	0

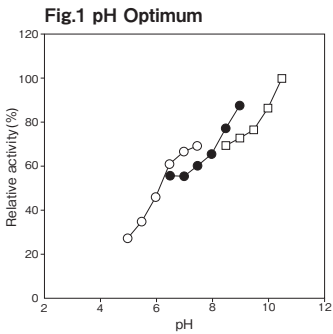
Table 2. Effect of metal ions on NADS II activity

Metal ion (1mM)	Relative activity (%)
None	100
NiCl ₂	1.1
BaCl ₂	106.0
SnCl ₂	99.8
AlCl ₃	95.6
CdSO ₄	2.4
MnCl ₂	18.2
CuCl ₂	3.6
ZnCl ₂	5.8
CoCl ₂	94.2
MgCl ₂	102.0
CaCl ₂	103.0
HgCl ₂	0.3

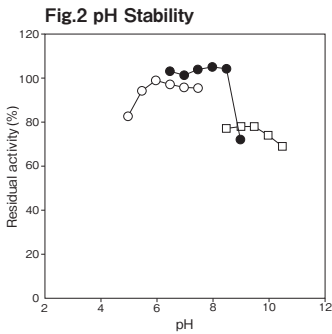
Table 3 Effect of detergents on NADS II activity

Detergent (0.1%)	Relative activity (%)
None	100
AdekatoI SO-120	108.2
SO-145	106.9
Brig 35	107.5
Cation DT-205	10.5
FB	11.8
Cetylpyridinium chloride	1.6
Sodium dodecyl sulfate	2.3
Tween 80	102.3
Cholic acid	105.6
Cetyltrimethyl ammonium chloride	4.2
Span 85	104.6
Sodium laurylbenzene sulfonate	6.5

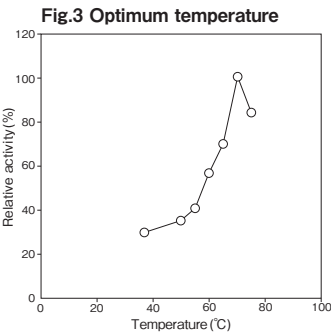
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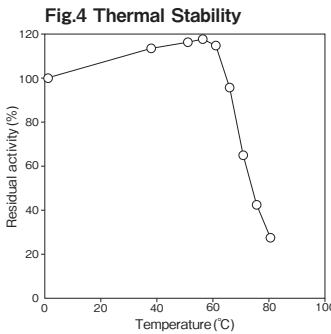
○ : 3,3-Dimethylglutarate-NaOH buffer
● : Tris-HCl buffer
□ : Glycine-NaOH buffer



37°C, 15min
○ : 3,3-Dimethylglutarate-NaOH buffer
● : Tris-HCl buffer
□ : Glycine-NaOH buffer



pH 8.0
100 mM Tris-HCl buffer

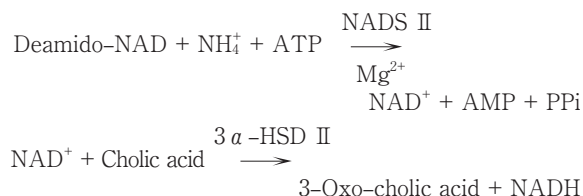


pH 7.5, 10min.
100 mM Tris-HCl buffer

Assay

■ Principle

The assay is based on the increase in absorbance at 340 nm as the formation of NADH proceeds in the following reactions:



NAD: Nicotinamide adenine dinucleotide

ATP: Adenosine triphosphate

■ Unit definition

One unit is defined as the amount of enzyme which converts 1 μ mole of deamido-NAD to NAD⁺ per minute at 37°C under the conditions specified in the assay procedure.

■ Reagents

- Reaction mixture

1 M Diethanolamine-HCl buffer pH 9.5	0.20 ml
20 mM Deamido-NAD solution	0.20 ml
0.2 M ATP solution pH 7.0	0.15 ml
0.1 M MgCl ₂ solution	0.30 ml
0.1 M NH ₄ Cl solution	0.30 ml
200 U/ml 3 α -HSD II solution	0.20 ml
50 mM Sodium cholate solution	0.40 ml
Distilled water	0.25 ml
- Reaction stopper

0.3 M EDTA solution pH 9.5	
EDTA: Ethylenediamine tetraacetic acid	
- Enzyme dilution buffer

20 mM Bicine-NaOH buffer pH 8.5	
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- Reagents

Diethanolamine:	
FUJIFILM Wako Pure Chemical Corporation	
Special grade #093-03115	
Deamido-NAD: Oriental Yeast Co., Ltd.	
ATP: Kyowa Hakko Co., Ltd.	
3 α -HSD II: Nagase Diagnostics Co., Ltd. #T-58	

Sodium cholate: Tokyo Kasei Kogyo Co., Ltd.

Special grade #C 0325

EDTA (2Na·2H₂O): KISHIDA CHEMICAL Co., Ltd.

#060-29133

Bicine: Dojindo Laboratories #347-03282

■ Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration to within 0.3-0.7 U/ml.

■ Procedure

- Pipette accurately 2.0 ml of reaction mixture into a small test tube and preincubate at 37°C.
- After 5 min, add exactly 50 μ l of enzyme solution and mix to start the reaction at 37°C.
※ In the case of a test blank, add 50 μ l of enzyme dilution buffer in place of enzyme solution.
- At 5 min after starting the reaction, add 1.0 ml of the reaction stopper to stop the reaction.
- Measure the absorbance at 340 nm.

$$\begin{array}{l} \text{Absorbance sample : } A_s \\ \text{blank : } A_b \\ 0.130 A_b \leq \Delta A = (A_s - A_b) \leq 0.390 A_b \end{array}$$

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\Delta A / 5}{6.3} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

6.3 : millimolar extinction coefficient of NADH at 340 nm
(cm² / μ mole)

5 : reaction time (min)

3.05 : final volume (ml)

0.05 : volume of enzyme solution (ml)

X : concentration of the sample in enzyme solution
(mg/ml)

Storage

Storage at -20°C in the presence of a desiccant is recommended.

NADS II 活性測定法 (Japanese)

I. 試薬液

- 反応試薬混合液

1.0 M DEA (Diethanolamine)-HCl 緩衝液	
pH9.5	0.20 ml
20 mM デアミド-NAD (NaNAD) 溶液 ¹⁾	0.20 ml
0.2 M ATP 溶液 pH7.0 ²⁾	0.15 ml
0.1 M MgCl ₂ 溶液	0.30 ml
0.1 M NH ₄ Cl 溶液	0.30 ml
200 U/ml 3 α -HSD II 溶液 ³⁾	0.20 ml
50 mM コール酸ナトリウム溶液	0.40 ml

精製水 0.25 ml

1): 20mM デアミド NAD 溶液

デアミド NAD 133 mg (純度換算) を精製水 10 ml で溶解する。

2): 0.2M ATP 溶液 pH7.0

ATP1.21g を精製水 8ml に溶解した後、4N NaOH で pH7.0 に調整し、精製水で全容を 10ml とする。

3): 200U/ml 3 α -HSD II 溶液

3 α -HSD II 2,000U を精製水 10ml で溶解する。

2. 反応停止液

0.3 M EDTA 溶液 pH9.5

3. 酵素溶解希釈用液

20 mM Bicine-NaOH 緩衝液 pH8.5

4. 試薬

DEA (ジエタノールアミン):

富士フイルム和光純薬製 特級 #093-03115

デアミド NAD: オリエンタル酵母製

ATP (アデノシン・三リン酸・2Na・3H₂O):

協和発酵製

3α-HSD II: ナガセダイアグノスティックス製 #T-58

コール酸ナトリウム:

東京化成工業製 特級 #C 0325

EDTA (エチレンジアミン四酢酸・2Na・2H₂O):

キシダ化学製 #060-29133

Bicine (ビシン): 同仁化学製 #347-03282

II. 酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液で溶解して全容 20ml とする。

その液を酵素溶解希釈用液で 0.3-0.7U/ml 濃度となるように適宜希釈する。

III. 測定操作法

1. 小試験管に反応試薬混合液 2.0ml を正確に分注し、37℃で予備加温する。

2. 5 分経過後、酵素試料液 50 μl を正確に加えて混和し、37℃で反応を開始する。

※盲検は酵素試料液の代わりに酵素溶解希釈用液 50 μl を加える。

3. 5 分経過後、反応停止液 1.0ml を加えて混和し、反応を停止する。

4. 340nm における吸光度を測定する。

求められた吸光度変化の試料液は As、盲検液は Ab とする。

$$0.130\text{Abs} \leq \Delta A = (A_s - A_b) \leq 0.390\text{Abs}$$

IV. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A / 5}{6.3} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

6.3: NADH の 340nm におけるミリモル分子吸光係数 (cm²/ μmole)

5 : 反応時間 (min)

3.05: 反応総液量 (ml)

0.05: 反応に供した酵素試料液量 (ml)

X : 酵素試料液中の検品濃度 (mg/ml)