

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 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 $4.3 \times 10^{-5}\text{M}$
 NH₃ $2.16 \times 10^{-3}\text{M}$
 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 (DI). Please refer to an information 3α-HSD II (T-58).

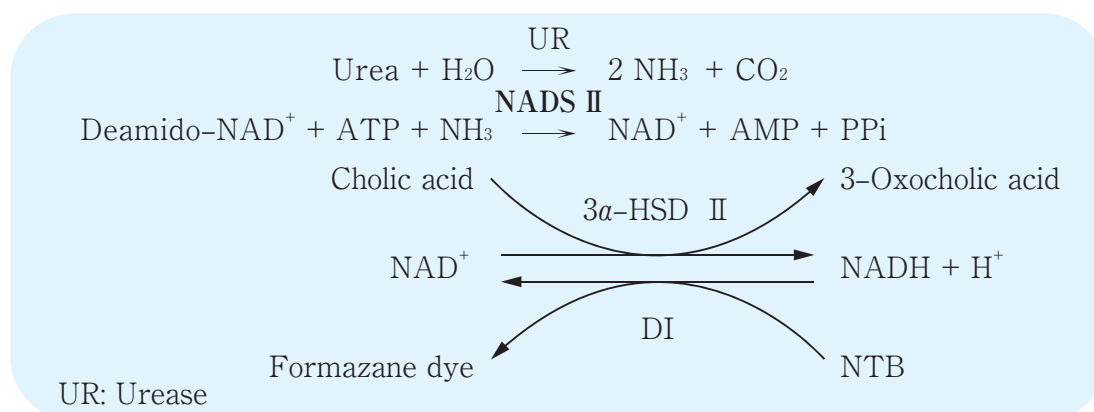


Table 1. Substrate specificity

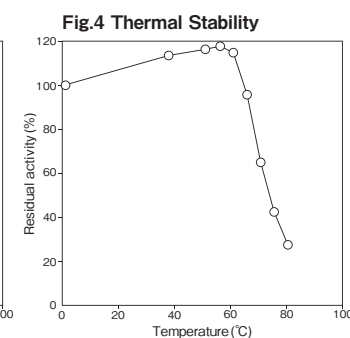
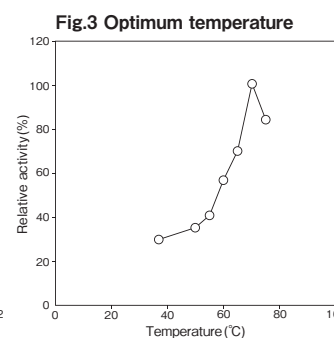
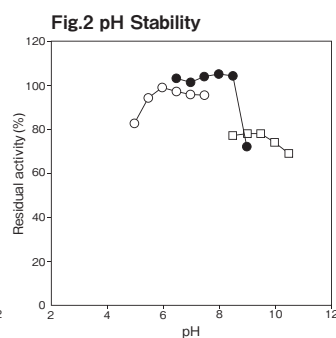
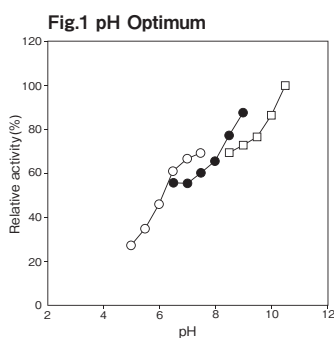
Substrate (1mM)	Relative activity (%)
NH ₄ Cl	100
L-aminoacids (Leu, Arg, Lys, Ileu, Tyr, Ala, Glu, Gln, Asn, Gly, Ser)	0
Azaserine	0
Urea	0
Uric acid	0
Creatinine	0
Creatine	0
Tris	0
Good's buffers	0

Table 3 Effect of detergents on NADS II activity

Detergent (0.1%)	Relative activity (%)
None	100
Adekamol 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

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



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

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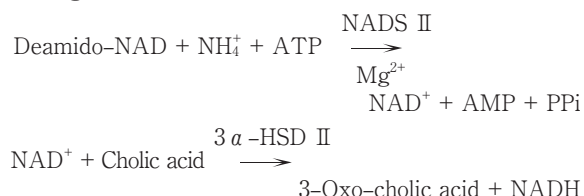
pH 8.0
 100 mM Tris-HCl buffer

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: Wako Pure Chemical Industries, Ltd.	
Special grade #093-03115	
Deamido-NAD: Oriental Yeast Co., Ltd.	
ATP: Kyowa Hakko Co., Ltd.	
3 α -HSD II: Asahi Kasei Pharma Corporation #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 10 minutes after starting the reaction, add 1.0 ml of the reaction stopper to stop the reaction.
- Measure the absorbance at 340 nm.

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

■ 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):
和光純薬工業製 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
3 α -HSD II: 旭化成ファーマ製 #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):
キシダ化学社製 #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 μ l 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 μ l 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 A_s、盲検液は A_b 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/ μ mole)

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)