

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).

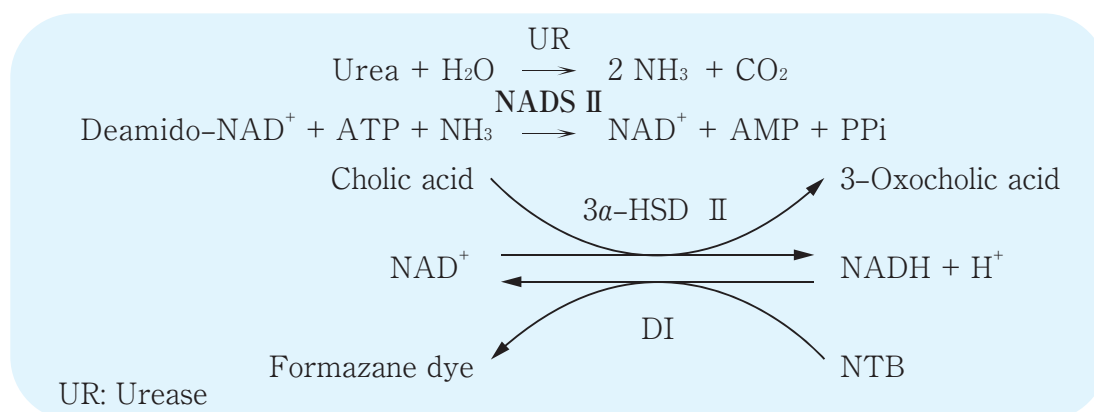


Table 1. Substrate specificity

| 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 |

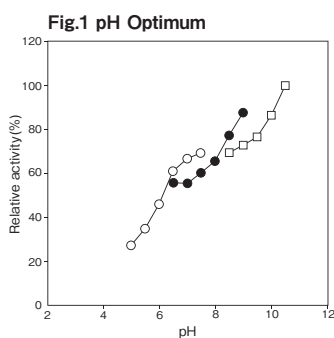


Fig.1 pH Optimum

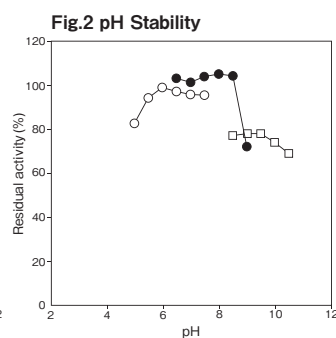


Fig.2 pH Stability

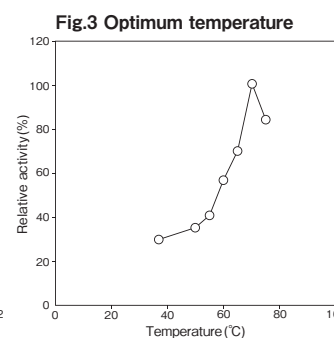


Fig.3 Optimum temperature

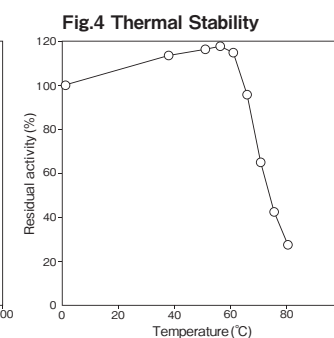


Fig.4 Thermal Stability

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

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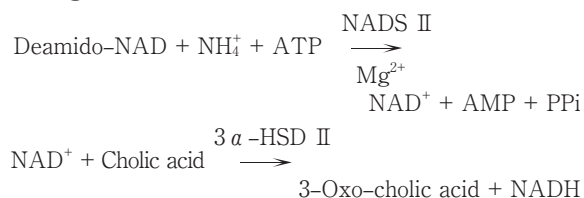
pH 8.0
100 mM Tris-HCl buffer

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 μmole 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_4Cl 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 | |
|---------------------------------|--|
- 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 μl of enzyme solution and mix to start the reaction at 37°C.
 - ※ In the case of a test blank, add 50 μl 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.

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

■ 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
($\text{cm}^2 / \mu\text{mole}$)

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_4Cl 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
3α-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 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 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/ 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)