NAD SYNTHETASE [NADS Ⅱ]

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

Deamido-NAD^+ + ATP + NH_3 ⟶ NAD^+ + AMP + PPi

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 2.4×10^{-5}M ATP 4.3×10^{-5}M NH_3 2.16×10^{-3}M	
Isoelectric point	: pH 5.2 ± 0.2	
Optimum pH	: 9.0-10.5	Figure 1
pH stability	: 6.0-9.0 (37℃, 15 min)	Figure 2
Optimum temperature	: 70℃ (Tris-HCl buffer)	Figure 3
Thermal stability	: Stable at 60℃ 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).

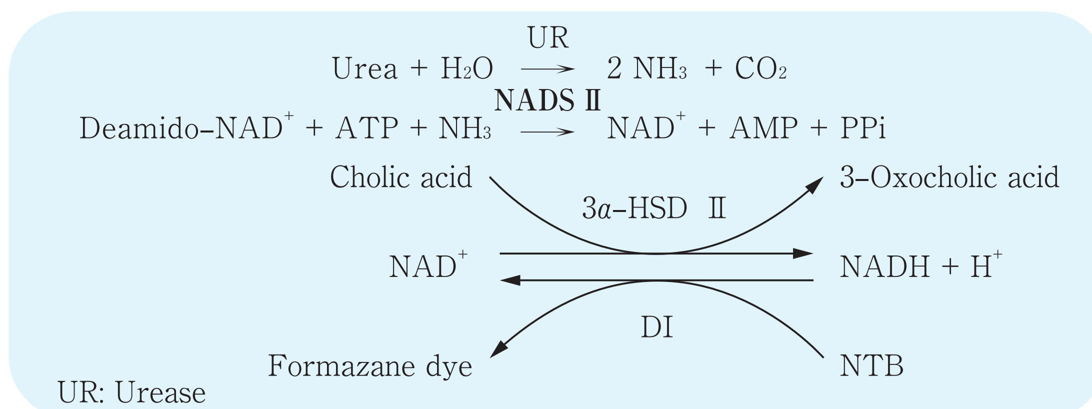

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Table 1. Substrate specificity

Substrate (1mM)	Relative activity (%)
NH_4Cl	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
Adekatol 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

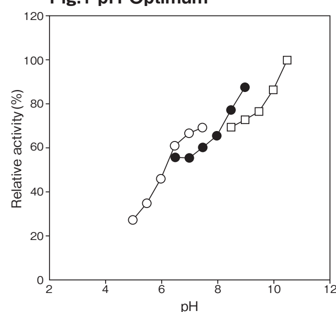


Fig.1 pH Optimum

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

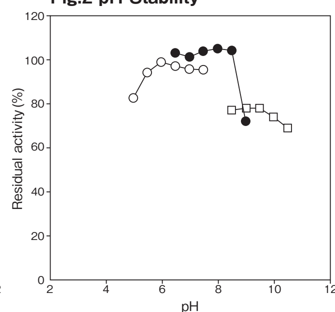


Fig.2 pH Stability

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

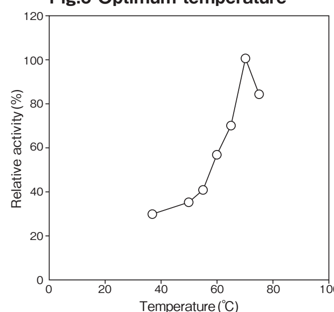


Fig.3 Optimum temperature

pH 8.0
100 mM Tris-HCl buffer

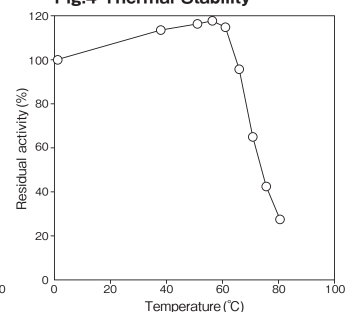


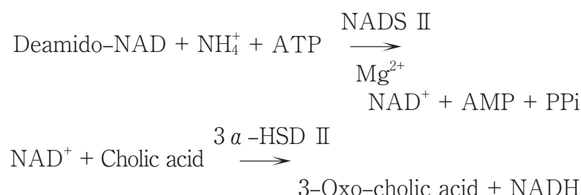
Fig.4 Thermal Stability

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: Nicotineamido 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℃ under the conditions specified in the assay procedure.

■ Reagents

1. 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 3α-HSD Ⅱ solution	0.20 ml
50 mM Sodium cholate solution	0.40 ml
Distilled water	0.25 ml

2. Reaction stopper
   0.3 M EDTA solution pH 9.5
   EDTA: Ethylenediamine tetraacetic acid
3. Enzyme dilution buffer
   20 mM Bicine-NaOH buffer pH 8.5
4. Reagents
   Diethanolamine:
   FUJIFILM Wako Pure Chemical Corporation
   Special grade #093-03115
   Deamido-NAD: Oriental Yeast Co., Ltd.
   ATP: Kyowa Hakko Co., Ltd.
   3α-HSD Ⅱ: Asahi Kasei Pharma Corporation #T-58
   Sodium cholate: Tokyo Kasei Kogyo Co., Ltd.
   Special grade #C 0325
   EDTA (2Na·$2H_2O$): 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

1. Pipette accurately 2.0 ml of reaction mixture into a small test tube and preincubate at 37℃.
2. After 5 min, add exactly 50 μl of enzyme solution and mix to start the reaction at 37℃.
   ※ In the case of a test blank, add 50 μl of enzyme dilution buffer in place of enzyme solution.
3. At 5 min after starting the reaction, add 1.0 ml of the reaction stopper to stop the reaction.
4. Measure the absorbance at 340 nm.
   Absorbance sample : As
   blank : Ab
   0.130Abs ≦ △A = (As − Ab) ≦ 0.390 Abs

■ Calculation

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

6.3 : millimolar extinction coefficient of NADH at 340 nm (cm^2/ μmole)
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℃ in the presence of a desiccant is recommended.

NADS Ⅱ　活性測定法（Japanese）

Ⅰ．試薬液

1. 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 3α-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 3α-HSD Ⅱ溶液
3α-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・3H_2O）:

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・2H_2O）:

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。

$$0.130\text{Abs} \leqq \Delta A = (As - Ab) \leqq 0.390\ \text{Abs}$$

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（cm^2/ 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）

N