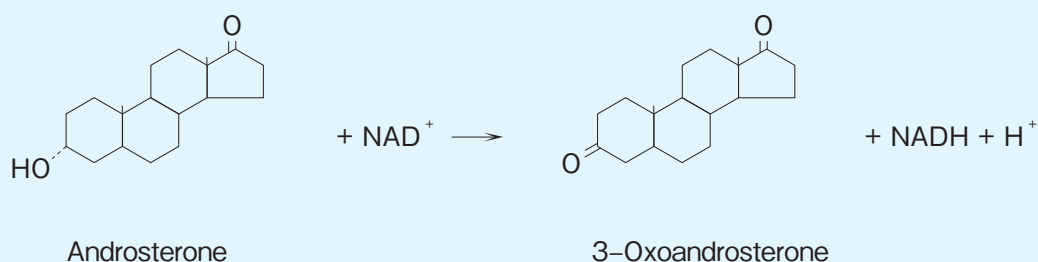


(Diagnostic Reagent Grade)

T-58

3 α -HYDROXYSTEROID DEHYDROGENASE [3 α -HSD II]

from *Pseudomonas* sp.
(3 α -Hydroxysteroid: NAD⁺ oxidoreductase, EC 1.1.1.50)



Preparation and Specification

Appearance : White amorphous powder, lyophilized
Specific activity : More than 30 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 41 kDa (gel filtration)	
Isoelectric point	: pH 4.8 ± 0.2	
Michaelis constant	: Androsterone 2.1 × 10 ⁻⁴ M	
Optimum pH	: 8.0-10.0	Figure 1
pH stability	: 6.0-10.0 (37°C, 10 min)	Figure 2
Optimum temperature	: 50°C (Phosphate buffer)	Figure 3
Thermal stability	: Stable at 50°C and below (pH 8.0, 10 min)	Figure 4
Effect of metal ions	: See Table 2	
Effect of detergents	: See Table 3	
Inhibitor	: MnCl ₂	

Applications for Diagnostic Test

This enzyme is useful for enzymatic cycling determination of **bile acid** when coupled with thio-NAD and NADH.

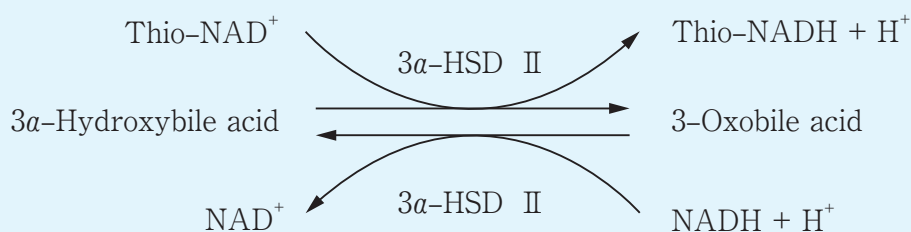


Table 1. Substrate specificity

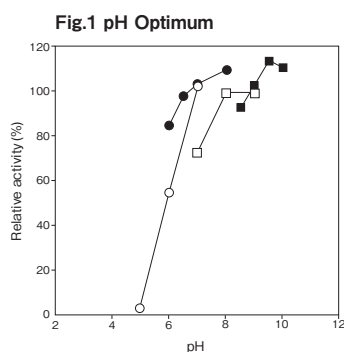
Substrate (1mM)	Relative activity (%)
Cholic acid	100
Androsterone	131
Deoxycholic acid	115
Chenodeoxycholic acid	89.0
Glycocholic acid	103
Taurocholic acid	99.0
Taurodeoxycholic acid	128

Table 2. Effect of metal ions on 3 α -HSD II activity

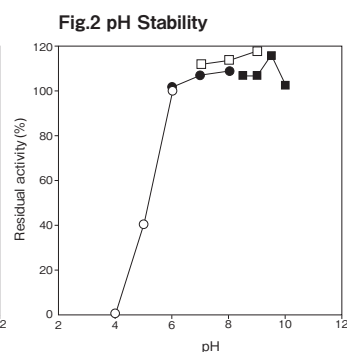
Metal ion (10mM)	Relative activity (%)
None	100
NaCl	105
KCl	102
LiCl	101
MgCl ₂	106
MnCl ₂	16.0
CaCl ₂	105

Table 3. Effect of detergents on 3 α -HSD II activity

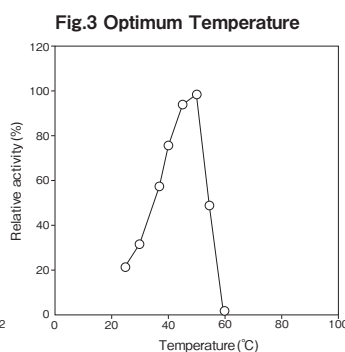
Detergent (0.1%)	Relative activity (%)
None	100
Triton X-100	71.0
Triton X-305	71.0
Triton X-114	71.0
Adekanol SO-120	104
Adekanol NP-720	75.0
Adekanol B-795	78.0
Emulgen B-66	75.0
Emulgen 911	76.0
Emulgen 709	84.0
Emulgen 810	50.0
Emulgen 109P	112
Rheodol 460	71.0
Rheodol TWL-103	72.0



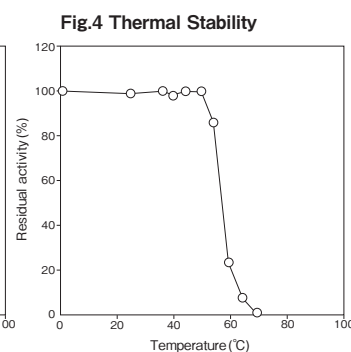
○ : 3,3-Dimethylglutarate-NaOH buffer
 ● : Phosphate buffer
 □ : Tris-HCl buffer
 ■ : Glycine-NaOH buffer



37°C, 10min
 ○ : Acetate buffer
 ● : Phosphate buffer
 □ : Tris-HCl buffer
 ■ : Glycine-NaOH buffer



pH 8.0
 20 mM Phosphate buffer

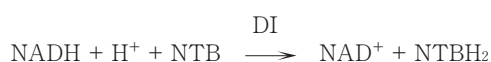
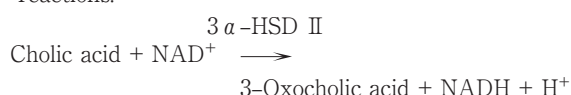


pH 8.0, 10 min.
 20 mM Phosphate buffer

Assay

Principle

The assay is based on the increase in absorbance at 550 nm as formazan dye is formed in the following reactions:



NAD: Nicotinamide adenine dinucleotide,

NTB: Nitroterazolum blue

DI: Diaphorase

Unit definition

One unit is defined as the amount of enzyme which oxidizes 1 μ mole of cholic acid to 3-oxocholeic acid per minute at 37°C under the conditions specified in the assay procedure.

Reagents

- Reaction mixture

10 mM NAD solution	0.05 ml
0.25% (W/V) NTB solution	0.05 ml
100 U/ml DI solution	0.025 ml
2% (W/V) Triton X-100 solution	0.10 ml
0.2 M Tris-HCl buffer pH 8.0	0.10 ml
Distilled water	0.175 ml
- 1): 100 U/ml DI solution
 Dissolve 100 U of DI with 1 ml of 10 mM Tris-HCl buffer pH 8.0.
- Substrate solution (20 mM Androsterone)
 Dissolve 23 mg of androsterone with 4 ml of methanol.
- Reaction stopper
 0.5% (W/V) Sodiumdodecyl sulfate (SDS) solution
- Enzyme dilution buffer
 10 mM Tris-HCl buffer pH 8.0
- Reagents
 NAD: NACALAI TESQUE, INC. #24334-84

NTB: Dojindo Laboratories #344-02033
DI: Nagase Diagnostics Co., Ltd. #T-06
Triton X-100: The Dow Chemical Company
Androsterone: Sigma Chemical Co. #A-9755
SDS (Sodium Dodecyl Sulfate) :

NACALAI TESQUE, INC. Extra pure #31606-75

■ 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 as required.

■ Procedure

1. Pipette accurately 0.5 ml of reaction mixture into a small test tube, then add 20 μ l of enzyme solution into the same test tube and preincubate at 37°C.
※ In the case of a test blank, add 20 μ l of enzyme dilution buffer in place of enzyme solution.
2. After 5 min, add exactly 25 μ l of substrate solution and mix to start the reaction at 37°C.
3. At 5 min after starting the reaction, add 2.5 ml of the reaction stopper to stop the reaction.
4. Measure the absorbance at 550 nm.
Absorbance sample : As
blank : Ab
 $\Delta A = (As - Ab) \leq 0.20$ Abs

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\Delta A/5}{16.7} \times \frac{3.045}{0.02} \times \frac{1}{X}$$

16.7 : millimolar extinction coefficient of NTB_{H2} at 550 nm
(cm²/ μ mole)

5 : reaction time (min)

3.045 : final volume (ml)

0.02 : 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.

References

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6. Mowszowicz, I. and Bardin, C. W. (1974) Steroids, **23**, 793-807.
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H

3 α -HSD II 活性測定法 (Japanese)

I. 試薬液

1. 反応試薬混合液
10mM NAD 溶液 0.05 ml
0.25% (W/V) NTB 溶液 0.05 ml
100U/ml DI 溶液¹⁾ 0.025 ml
2% (W/V) トリトン X-100 溶液 0.10 ml
0.2M トリス-HCl 緩衝液 pH8.0 0.10 ml
精製水 0.175 ml
1): 100U/ml DI 溶液
DI 100 単位 (U) を 10mM トリス-HCl 緩衝液 pH8.0 1ml で溶解する。
2. 基質溶液 (20mM アンドロステロン溶液)
アンドロステロン 23mg を MeOH4ml で溶解する。
3. 反応停止液
0.5% (W/V) SDS 溶液
4. 酵素溶解希釈用液
10mM トリス-HCl 緩衝液 pH8.0
5. 試薬
NAD (ニコチンアミドアデニンジヌクレオチド):
ナカライテスク製 #24334-84
NTB (ニトロテトラゾリウムブルー):
同仁化学製 #344-02033
DI (ジアフォラーゼ) : ナガセダイアグノスティクス製 #T-06
トリトン X-100: Dow Chemical 製
アンドロステロン: シグマ製 #A-9755
SDS (ドデシル硫酸ナトリウム):
ナカライテスク製 一級 #31606-75

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 0.5ml を正確に分注し、後に酵素試料液 20 μ l を正確に分注して 37°C で予備加温する。
※盲検は酵素試料液の代わりに酵素溶解希釈用液 20 μ l を加える。
2. 5 分経過後、基質溶液 25 μ l を正確に加えて混和し、37°C で反応を開始する。
3. 5 分経過後、反応停止液 2.5ml を正確に加えて混和し、反応を停止する。
4. 550nm における吸光度を測定する。
求められた吸光度を試料液は As、盲検液は Ab とする。
 $\Delta A = (As - Ab) \leq 0.20$ Abs

IV. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A/5}{16.7} \times \frac{3.045}{0.02} \times \frac{1}{X}$$

16.7 : NTB_{H2} の 550nm におけるミリモル分子吸光係数
(cm²/ μ mole)

5 : 反応時間 (min)

3.045 : 反応総液量 (ml)

0.02 : 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)