



**3-alpha Hydroxysteroid  
Dehydrogenase  
Microplate Assay Kit  
User Manual**

**Catalog # CAK1170**

(Version 1.2A)

Detection and Quantification of 3-alpha Hydroxysteroid  
Dehydrogenase (3 $\alpha$ -HSD) Activity in Urine, Serum, Plasma, Other  
biological fluids Samples.

**For research use only. Not for diagnostic or therapeutic procedures.**

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## I. INTRODUCTION

3-alpha Hydroxysteroid Dehydrogenase (EC 1.1.1.50) belongs to the family of oxidoreductases, specifically those acting on the CH-OH group of donor with NAD<sup>+</sup> or NADP<sup>+</sup> as acceptor, more specifically it is part of the group of hydroxysteroid dehydrogenases. The systematic name of this enzyme class is 3alpha-hydroxysteroid: NAD(P)<sup>+</sup> oxidoreductase (B-specific). Other names in common use include hydroxyprostaglandin dehydrogenase, 3alpha-hydroxysteroid oxidoreductase, and sterognost 3alpha. This enzyme participates in 3 metabolic pathways: bile acid biosynthesis, c21-steroid hormone metabolism, and androgen and estrogen metabolism.

3-alpha Hydroxysteroid Dehydrogenase Microplate Assay Kit is a sensitive assay for determining 3-alpha Hydroxysteroid Dehydrogenase activity in various samples.

3-alpha Hydroxysteroid Dehydrogenase reacts with bile acids, converting NAD to NADH, the color intensity at 340 nm is linear to the 3-alpha Hydroxysteroid Dehydrogenase activity in the sample.

## II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

**Note:**

**Substrate:** add 1 ml Assay Buffer to dissolve before use.

**Standard:** add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400  $\mu\text{mol/L}$ .

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

#### **IV. SAMPLE PREPARATION**

1. For urine, serum, or other biological fluids samples

Detect directly.

## V. ASSAY PROCEDURE

Warm all reagents to 37 °C before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 µl	--
Distilled water	--	--	200 µl
Reaction Buffer	180 µl	--	--
Substrate	10 µl	--	--
Sample	10 µl	--	--
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

### Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

## VI. CALCULATION

**Unit Definition:** One unit of 3 $\alpha$ -HSD activity is defined as the enzyme produces 1 nmol of NADH per minute.

1. According to the protein concentration of sample

$$\begin{aligned} 3\alpha\text{-HSD (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample(130S)}} - OD_{\text{Sample(10S)}}) / (OD_{\text{Standard}} - \\ &\quad OD_{\text{Blank}}) / (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 4000 \times (OD_{\text{Sample(130S)}} - OD_{\text{Sample(10S)}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the volume of serum or plasma

$$\begin{aligned} 3\alpha\text{-HSD (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample(130S)}} - OD_{\text{Sample(10S)}}) / (OD_{\text{Standard}} - \\ &\quad OD_{\text{Blank}}) / V_{\text{Sample}} / T \\ &= 4000 \times (OD_{\text{Sample(130S)}} - OD_{\text{Sample(10S)}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \end{aligned}$$

$C_{\text{Standard}}$ : the standard concentration, 400  $\mu\text{mol/L}$  = 400 nmol/ml;

$V_{\text{Standard}}$ : the volume of standard, 200  $\mu\text{l}$  = 0.2 ml;

$C_{\text{Protein}}$ : the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

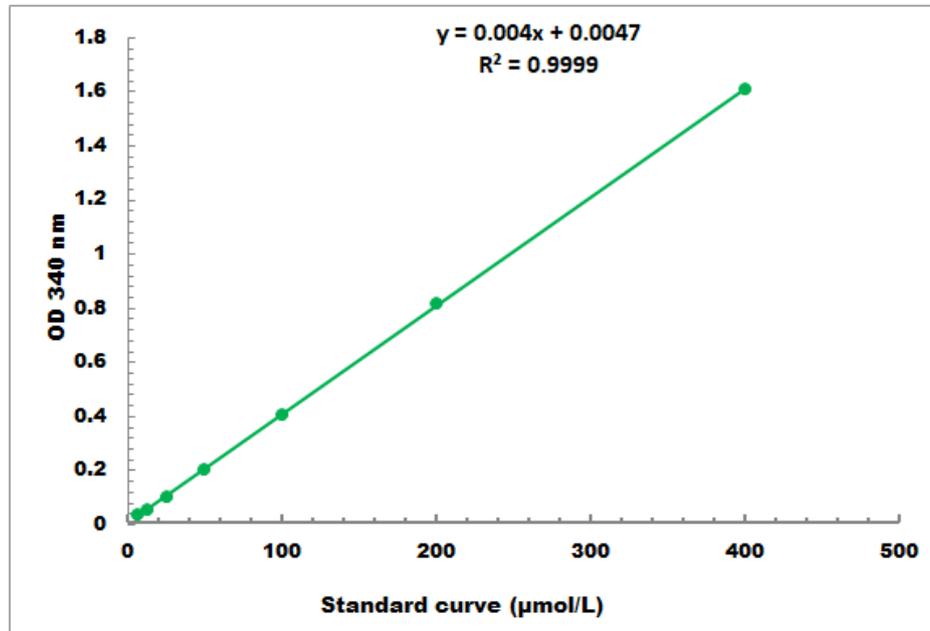
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

## VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 µmol/L - 400 µmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES