



3-alpha Hydroxysteroid Dehydrogenase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1170

(Version 1.6C)

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

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

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	4
IV. REAGENT PREPARATION.....	5
V. SAMPLE PREPARATION.....	6
VI. ASSAY PROCEDURE.....	7
VII. CALCULATION.....	8
VIII.TYPICAL DATA.....	9

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⁺ or NADP⁺ 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)⁺ 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 Activity Colorimetric 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 450 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	30mlx 4	4 °C
Reaction Buffer	10 mlx 1	4 °C
Substrate	Powder x 1	-20 °C
Dye ReagentA	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 400 µmol/L standard top solution by adding 0.2 ml stock solution into 0.8 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25 µmol/L.

Substrate: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use. Keep in dark for immediate use or store at -20°C for 1 week after reconstitution.

Dye Reagent A: Add 9 ml distilled water to dissolve before use, mix. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use. Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

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

Detect directly.

VI. ASSAY PROCEDURE

Warm all reagents to 37°C before use.

Add following reagents into the microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Sample	10 µl	--	--	--	
Standard	--	--	100 µl	--	
Positive Control					10 µl
Reaction Buffer	80 µl	80 µl	--	--	80 µl
Substrate	10 µl	10 µl	--	--	10 µl
Distilled water	--	10 µl	--	100 µl	
Mix.					
Dye Reagent A	90 µl	90 µl	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, incubate at room temperature for 5 minutes, record absorbance measured at 450 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of 3 α -HSD activity is defined as the enzyme produces 1 μ mol of NADH per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein}: the protein concentration of sample, mg/mL

C_{Standard}: the concentration of standard, μ mol/mL

V_{Standard}: the volume of standard in assay procedure, mL

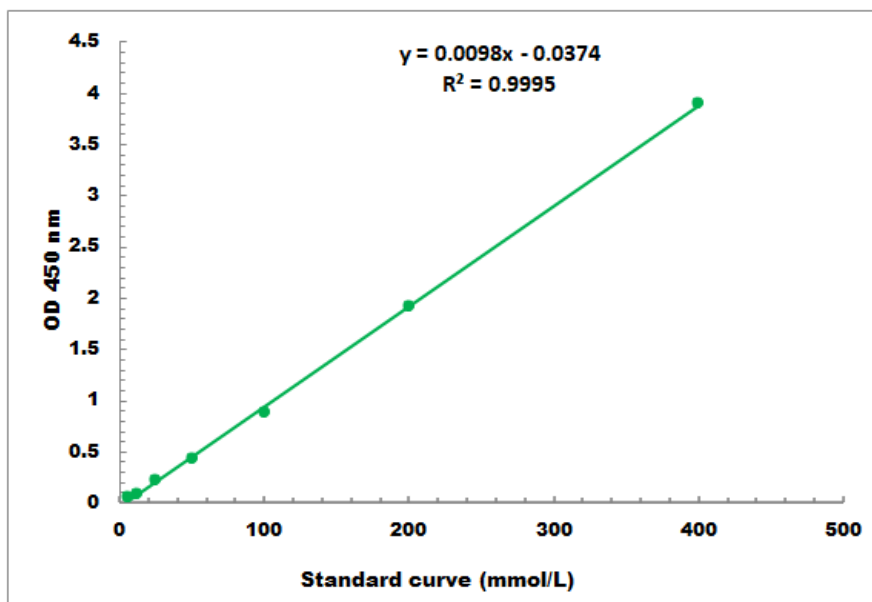
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

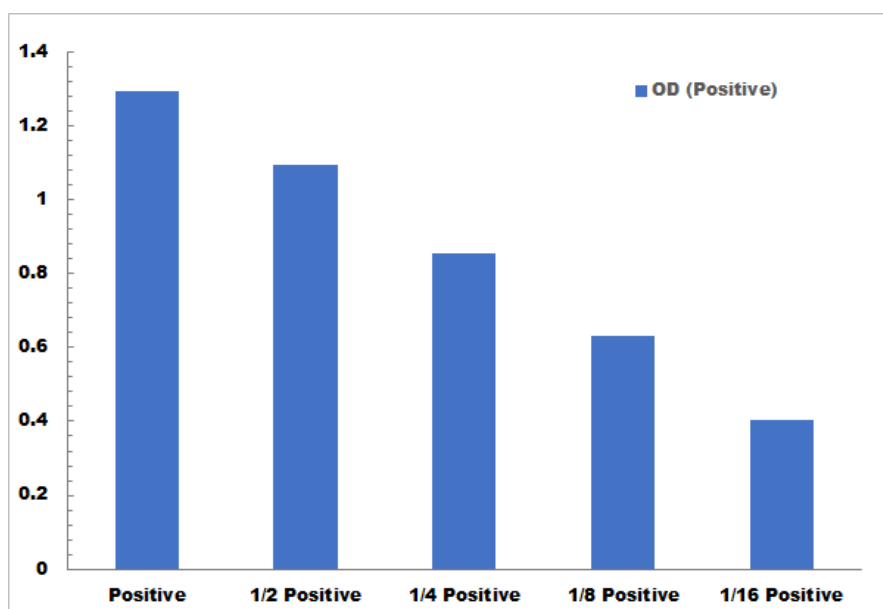
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 4 μ mol/L -400 μ mol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration