3-alpha Hydroxysteroid Dehydrogenase Microplate Assay Kit User Manual

Catalog # CAK1170

(Version 1.5C)

Detection and Quantification of 3-alpha Hydroxysteroid Dehydrogenase (3α-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+ 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 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	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Dye Reagent A	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	

Note:

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

Dye Reagent A: add 9 ml distilled water to dissolve before use, mix, store at 4°C.

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 µmol/L.

Positive Control: add 1 ml distilled water to dissolve before use.

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. 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	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:

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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

$$3\alpha\text{-HSD (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.8 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the volume of serum or plasma

$$3\alpha\text{-HSD (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.8 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 0.4 μmol/ml;

$V_{Standard}$: the volume of standard, 100 μl = 0.1 ml;

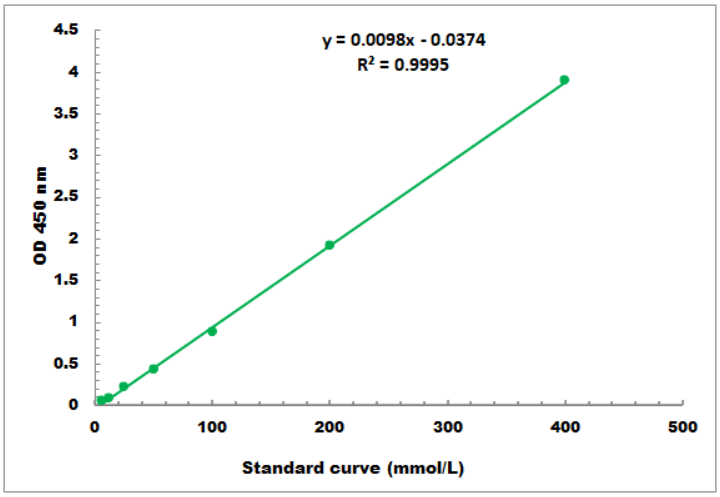
$C_{Protein}$: the protein concentration, mg/ml;

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

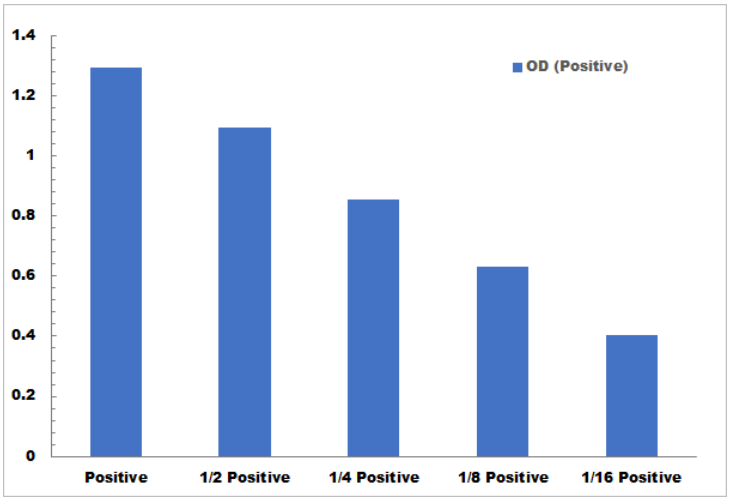
T: the reaction time, 5 minutes.

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



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES