3-alpha Hydroxysteroid Dehydrogenase Microplate Assay Kit

Cat #: orb707346 (manual)

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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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.

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.

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

SAMPLE PREPARATION

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

Detect directly.

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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.

CALCULATION

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

1. According to the protein concentration of sample

$$3\alpha\text{-HSD (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$
$$= 800 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the volume of serum or plasma

$$3\alpha\text{-HSD (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$
$$= 800 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 400 nmol/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.

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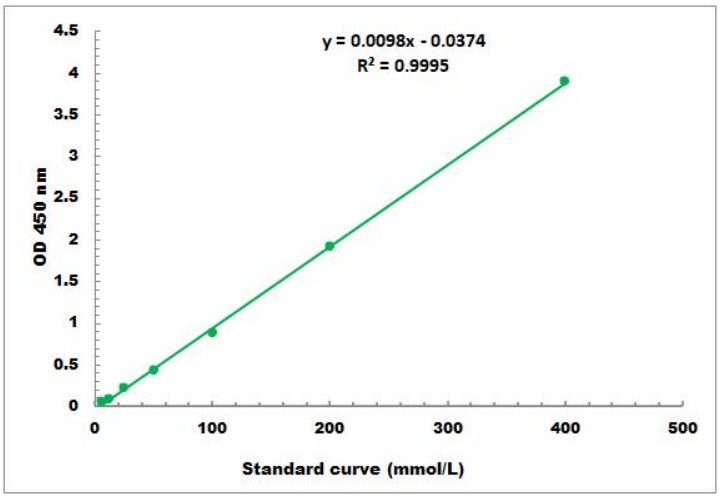
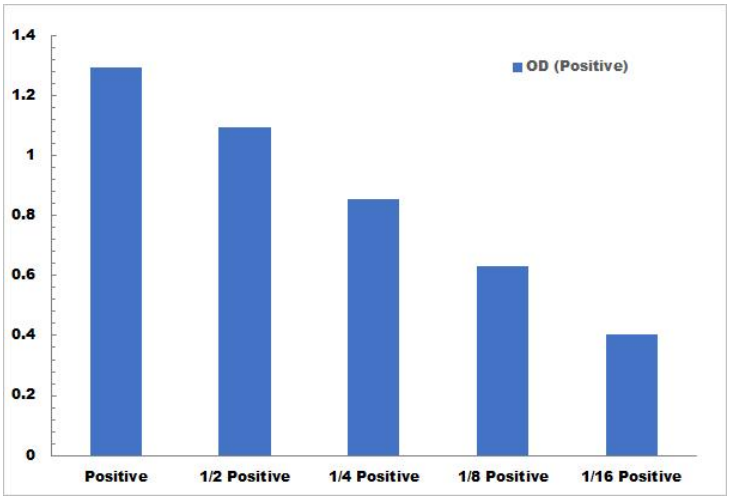
TYPICAL DATA

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

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