Diagnostics Biochem Canada

17α-Hydroxyprogestone (17α-OHP) ELISA

REF CAN-P-400

ASSAY PROCEDURE

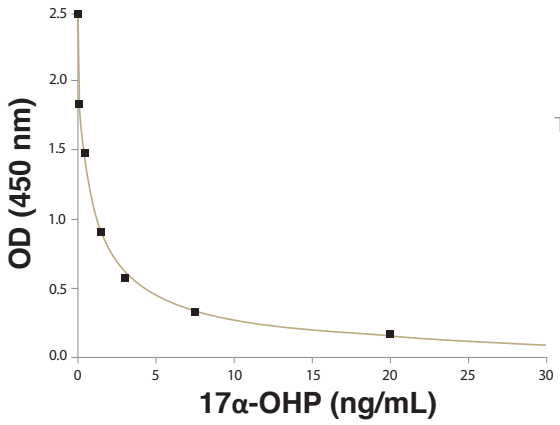
- Bring kit components to room temperature. Prepare working solutions.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 150 µL of 17α-OHP-HRP conjugate.
- Gently shake the microplate by hand for 10 seconds.
- Incubate for 1 hour at room temperature (without shaking).
- Wash 3 times. Pipette 150 µL of TMB substrate into each well.
- Gently shake the microplate by hand for 10 seconds.
- Incubate for 15–20 minutes at room temperature (without shaking).
- Pipette 50 µL of stopping solution into each well. Gently shake the microplate for 10 seconds.
- Read the plate on a microplate reader at 450 nm.

17α-hydroxyprogesterone is produced by the adrenal cortex and gonads. 17α-OHP has little progestational activity, but has intense clinical interest because it is the immediate precursor to 11-desoxycortisol, which is produced by the 21-hydroxylation of 17α-OHP. Measurement of 17α-OHP is, consequently, a useful indirect indicator of 21-hydroxlase activity.

In congenital 21-hydroxylase deficiency, the most common variety of congenital adrenal hyperplasia (CAH), 17α-OHP is secreted in abundant excess. Measurement of 17α-OHP is therefore valuable in the initial diagnosis of CAH.

PRINCIPLE OF THE TEST

DBC's 17α-Hydroxyprogesterone (17α-OHP) ELISA is a competitive enzyme immunoassay. Competition occurs between the antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. After samples and conjugate have been incubated for one hour, washing of the microplate removes unbound materials and an enzyme substrate that generates colour is added. The enzymatic reaction is terminated by addition of stopping solution. The optical density, measured with a microplate reader, is inversely proportional to the concentration of 17α-OHP in the sample. A set of standards is used to plot a standard curve from which the concentration of 17α-OHP in patient samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 60 samples of the blank and a low concentration sample in two independent experiments and it was calculated as follows:

LoD = μB + 1.645σB + 1.645σS

Where σB and σS are the standard deviation of the blank and a low value sample and μB is the mean value of the blank. The LoD was determined to be **0.051 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the 17α-OHP ELISA kit with 17α-OHP cross-reacting at 100%.

Steroid	% Cross-Reactivity
17α-Hydroxyprogesterone	100
Progesterone	1.7
11-Desoxycortisol	< 0.25
DHEA	< 0.25
DHEAS	< 0.25
Cortisol	< 0.25
Cholesterol	< 0.25
Pregnenolone	< 0.25
Pregnenolone-SO4	< 0.25
Prednisone	< 0.25

INTERFERENCE

Serum samples with varying levels of 17α-OHP were tested after spiking with potential interfering substances at levels that exceed the highest found concentration in serum. To calculate the % of interference, results were compared to the same serum samples with no extra substances added. The following substances were tested and did not show significant interference in the assay: hemoglobin up to 2 g/L; bilirubin conjugated and free up to 10 mg/dL; triglycerides up to 5 mg/mL; rheumatoid factor up to 1.2 IU/mL; HAMAS 1.2 μg/mL.

COMPARATIVE STUDIES

The DBC 17α-OHP ELISA kit (y) was compared to a higher level test (RIA) (x). The comparison of 49 serum samples yielded the following linear regression results:

y = 0.83x + 0.13, r = 0.99

PRECISION

Six samples were assayed in duplicate in 40 independent experiments ran by two operators during 10 days. The results (in ng/mL) are tabulated below:

Sample	Mean	Within Run SD	Within Run CV	Total SD	Total CV
1	0.685061	0.026898	3.9%	0.107806	15.7%
2	4.30577	0.19803	4.6%	0.63436	14.7%
3	7.14774	0.27497	3.8%	0.86208	12.1%
4	8.64947	0.42710	4.9%	1.04203	12.0%
5	10.14976	0.39541	3.9%	1.37120	13.5%
6	15.0621	0.6751	4.5%	1.6217	10.8%

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Recovery %
1 1:2 1:5 1:10	15.75 9.14 4.03 1.69	- 116 128 107
2 1:2 1:5 1:10	13.55 6.02 2.61 1.10	- 89 96 81
3 1:2 1:5 1:10	21.88 12.66 5.54 2.55	- 116 127 116

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Median (ng/mL)	95% Confidence Range (ng/mL)
Children 3–12 years old	80	0.31	0.051–2.35
Children 13–17 years old	80	0.72	0.13–1.85
Women < 40 years old	120	0.93	0.27–2.54
Women > 60 years old	120	0.43	0.094–1.02
Men 20–59 years old	240	1.60	0.37–2.87

Ordering Information: **REF CAN-P-400**

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