Diagnostics Biochem Canada Inc.
Manufacturing Innovative IVD for the World Since 1973

17α-HYDROXYPROGESTERONE (17α-OHP) ELISA

EU: CE IVD CAN/USA: IVD

REF: CAN-P-400 Version: 4.1 (COMB)
Effective: October 17, 2023

INTENDED USE

For the direct quantitative determination of 17α-hydroxyprogesterone (17α-OHP) in human serum by an enzyme immunoassay.

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.

CLINICAL APPLICATIONS

The steroid 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.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is intended for in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Avoid microbial contamination of reagents.
5. A calibrator curve must be established for every run.
6. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
7. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing or inadequate reagent storage.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
10. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
11. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
12. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator and control.
15. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
16. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of 17α-OHP in human serum. The kit is not calibrated for the determination of 17α-OHP in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.05 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower temperature for longer time. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 50, 150 and 350 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater

REAGENTS PROVIDED

1. Anti-17α-OHP Antibody-Coated Microplate — Ready To Use
- Contents: One 96-well (12x8) rabbit antibody-coated microplate in a resealable pouch with desiccant.
- Storage: Refrigerate at 2–8°C
- Stability: 12 months or as indicated on label.

2. 17α-OHP-Horseradish Peroxidase (HRP) Conjugate — Ready To Use
- Contents: 17α-OHP-HRP conjugate in a protein-based buffer with a non-mercury preservative.
- Volume: 20 mL/bottle
- Storage: Refrigerate at 2–8°C
- Stability: 12 months or as indicated on label.

3. 17α-OHP Calibrators — Ready to Use
- Contents: Seven vials containing 17α-OHP in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of 17α-OHP.

* Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 ng/mL	2.0 mL
Calibrator B	0.15 ng/mL	1.0 mL
Calibrator C	0.5 ng/mL	1.0 mL
Calibrator D	1.5 ng/mL	1.0 mL
Calibrator E	3 ng/mL	1.0 mL
Calibrator F	7.5 ng/mL	1.0 mL
Calibrator G	20 ng/mL	1.0 mL

- Storage: Refrigerate at 2–8°C
- Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Controls — Ready to Use
- Contents: Two vials containing 17α-OHP in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of 17α-OHP. Refer to vial labels for the acceptable range.
- Volume: 1.0 mL/vial
- Storage: Refrigerate at 2–8°C
- Stability: 12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate — Requires Preparation X10
- Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
- Volume: 50 mL/bottle
- Storage: Refrigerate at 2–8°C
- Stability: 12 months or as indicated on label.
- Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. TMB Substrate — Ready To Use
- Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
- Volume: 16 mL/bottle
- Storage: Refrigerate at 2–8°C
- Stability: 12 months or as indicated on label.

7. Stopping Solution — Ready To Use
- Contents: One bottle containing 1M sulfuric acid.
- Volume: 6 mL/bottle
- Storage: Refrigerate at 2–8°C
- Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Specimen Pretreatment: None.

All reagents must reach room temperature before use.

Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all the kit components have reached room temperature, mix gently by inversion. Prepare the working wash buffer (see wash buffer concentrate under the section “Reagents Provided”).
2. Remove the required number of microplate strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 25 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 150 µL of the 17α-OHP-HRP conjugate into each well. (We recommend using a multi-channel pipette.)
5. Gently shake the microplate by hand for 10 seconds to ensure complete mixing of the conjugate solution with samples, controls and standards.
6. Incubate for 1 hour at room temperature (do not shake).
7. Wash the wells **3 times** with 350 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of an automated microplate washer is recommended.)
8. Pipette 150 µL of TMB substrate into each well at timed intervals. Gently shake the microplate by hand for 10 seconds.
9. Incubate for 15–20 minutes at room temperature (do not shake), or until calibrator A attains dark blue colour for desired OD.
10. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 8. Gently shake the microplate by hand for 10 seconds.
11. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

CALCULATIONS

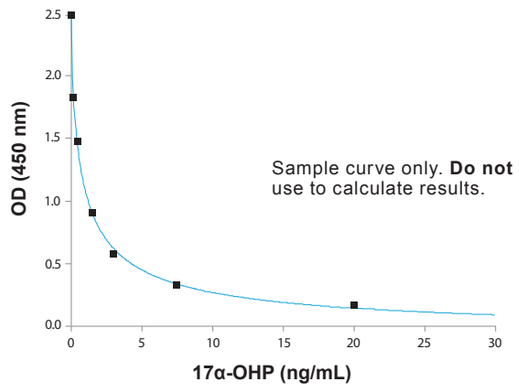
1. Calculate the mean optical density of each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve with immuno-assay software to generate the control and sample concentration results or draw a calibration curve on semi- log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis and read the concentration of controls and samples off the calibrator curve.
3. If the sample reads more than 20 ng/mL, then dilute it with calibrator A at a dilution of no more than 1:10. The result obtained must be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	OD 1	OD 2	Mean OD	Value (ng/mL)
A	2.528	2.438	2.483	0
B	1.808	1.856	1.832	0.15
C	1.475	1.481	1.478	0.5
D	0.903	0.915	0.909	1.5
E	0.584	0.572	0.578	3
F	0.328	0.340	0.334	7.5
G	0.165	0.166	0.166	20
Unknown	0.414	0.416	0.415	5.53

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.

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

COMPARITIVE 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

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

REFERENCES

1. Yeo KH, et al. An Automated Solid-Phase 17α-Hydroxy-progesterone ELISA Method Using a Microtiter Plate. *Ann Clin Biochem.* 1988; 25(Pt 3):293–7.
2. Hofman LF, et al. Direct Solid-Phase Radioimmunoassay for Screening 17α-Hydroxyprogesterone in Whole-Blood Samples from Newborns. *Clin Chem.* 1985; 31(7):1127–30.
3. Sippel WG, et al. Plasma Levels of Aldosterone, Cortico-sterone, 11-Deoxycorticosterone, Progesterone, 17-hydroxy-progesterone, Cortisol, and Cortisone During Infancy and Childhood. *Pediatr Res.* 1980; 14(1):39–46.
4. Thorneycroft IH, et al. The Relation of Serum 17-Hydroxy-progesterone and Estradiol 17-beta Levels During the Human Menstrual Cycle. *Am J Obstet Gynecol.* 1971; 111: 947–51.

DBC-Diagnostics Biochem Canada Inc.
384 Neptune Crescent
London, Ontario, Canada N6M 1A1
Tel: (519) 681-8731
Fax: (519) 681-8734
e-mail: dbc@dbc-labs.com
www.dbc-labs.com
An ISO 13485 Registered Company

EC REP **MedEnvoy Global B.V.**
Prinses Margrietplantsoen 33, Suite 123
2595AM The Hague
The Netherlands

SYMBOLS

CE European Comformity
IVD In vitro diagnostic device
Consult instructions for use
Contains sufficient for <n> tests
Storage Temperature
Legal Manufacturer
Use by
REF Catalogue Number
EC REP Authorized representative
LOT Lot number
X # Dilute 1: # Before use