# 17α-Hydroxyprogesterone (17α-OHP) ELISA Assay Kit

Catalog Number:
P1731-K01 (1 x 96 wells)
*For Research Use Only. Not for use in diagnostic procedures.*
v. 1.1 (25 SEP 23)

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## INTENDED USE

The Eagle Biosciences 17α-hydroxyprogesterone (17α-OHP) ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the direct quantitative determination of 17α-hydroxyprogesterone (17α-OHP) in human serum. The Eagle Biosciences 17α-hydroxyprogesterone (17α-OHP) ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

## INTRODUCTION

The steroid 17α-hydroxyprogesterone is produced by the adrenal cortex and gonads. 17α-OHP has little progestational activity, but it is of intense clinical interest because it is the immediate precursor to 11-desoxycortisol (Cpd-S). Cpd-S is produced by the 21-hydroxylation of 17α-OHP. Measurement of 17α-OHP is 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 ASSAY

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled 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. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed is inversely proportional to the concentration of 17α-OHP in the sample. A s et of standards is used to plot a standard curve from which the amount of 17α-OHP in patient samples and controls can be directly read.

## PROCEDURAL CAUTIONS AND WARNINGS

1. 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.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. 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.

10. The substrate solution (TMB) is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to 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 saliva samples. The use of any other reagent may lead to false results.
5. This kit is for research use only and should not be used for diagnostic procedures.

**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 nonreactive 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. No test method however, 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 0.1 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 if the analyses are to be done at a later date. 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 50, 100, 150 and 300 µL
2. Disposable pipette tips

3. Distilled or deionized water
4. Plate shaker
5. Automatic Microplate Washer
6. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

**REAGENTS PROVIDED**

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

2. **(HRP) Conjugate Concentrate** — Requires Preparation X100
   Contents: 17α-OHP-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 0.3 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation: Dilute 1:101 in assay buffer before use (eg. 20 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 180 µL of HRP in 18 mL of assay buffer. Discard any that is left over.

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/Vial |
|---|---|---|
| Calibrator A | 0 ng/mL | 2.0 mL |
| Calibrator B | 0.15 ng/mL | 0.6 mL |
| Calibrator C | 0.5 ng/mL | 0.6 mL |
| Calibrator D | 1.5 ng/mL | 0.6 mL |
| Calibrator E | 3 ng/mL | 0.6 mL |
| Calibrator F | 7.5 ng/mL | 0.6 mL |
| Calibrator G | 20 ng/mL | 0.6 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: 0.6 mL/vial

Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vial 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. **Assay Buffer** – Ready to Use
   Contents: One bottle containing 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.

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

8. **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 kit components have reached room temperature, mix gently by inversion.
2. Prepare working solution of the HRP conjugate and Wash Buffer.
3. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator. (see recommended assay layout)
4. Pipette 50 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.

5. Pipette 150 µL of the HRP conjugate working solution into each well. (We recommend using a multichannel pipette.)
6. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
7. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
8. Pipette 150 µL of TMB substrate into each well at timed intervals.
9. Incubate on a plate shaker for 10–15 minutes at room temperature (or until calibrator A attains dark blue color for desired OD).
10. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 7.
11. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

## CALCULATIONS

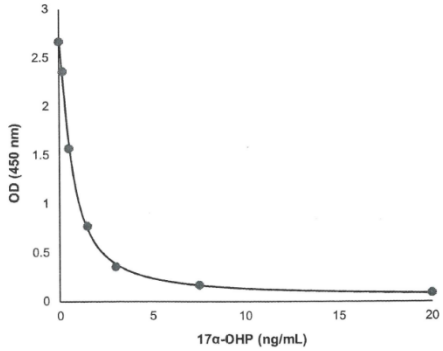
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. The Immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 20 ng/mL, then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

## TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

| Calibrator | Mean OD | % Binding | Value (ng/mL) |
|---|---|---|---|
| A | 2.665 | 100 | 0 |
| B | 2.361 | 89 | 0.15 |
| C | 1.573 | 59 | 0.5 |
| D | 0.772 | 29 | 1.5 |
| E | 0.353 | 13 | 3 |
| F | 0.166 | 6 | 7.5 |
| G | 0.089 | 3 | 20 |
| Unknown | 0.567 | - | 1.99 |

## TYPICAL CALIBRATOR CURVE



## PERFORMANCE CHARACTERISTICS

### SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the 17α-OHP ELISA kit is **0.11 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 | 0.25 |
| 11-Desoxycortisol | 0.77 |
| DHEA | < 0.1 |
| Corticosterone | < 0.1 |
| Cholesterol | < 0.1 |
| Pregnenolone | < 0.1 |
| Pregnenolone-SO4 | < 0.1 |
| Prednisone | < 0.1 |

### INTRA-ASSAY PRECISION

Three samples were assayed 20 times, on the same calibrator curve. The results (in ng/mL) are tabulated below:

| Sample | Mean | SD | CV % |
|---|---|---|---|
| 1 | 1.91 | 0.096 | 5.0 |
| 2 | 5.68 | 0.287 | 5.1 |
| 3 | 10.48 | 0.602 | 5.7 |

### INTER-ASSAY PRECISION

Three samples were assayed 10 times over a period of four weeks. The results (in ng/mL) are tabulated below:

| Sample | Mean | SD | CV % |
|---|---|---|---|
| 1 | 1.69 | 0.116 | 6.8 |
| 2 | 5.69 | 0.487 | 8.6 |
| 3 | 9.38 | 0.464 | 4.9 |

## RECOVERY

Spiked samples were prepared by adding defined amounts of 17α-OHP to three patient serum samples. The results (in ng/mL) are tabulated below:

| Sample | Obs. Result | Exp. Result | Recovery % |
|---|---|---|---|
| 1 Unspiked | 1.82 | - | - |
| + 0.15* | 2.61 | 2.75 | 95 |
| + 0.4* | 4.66 | 4.8 | 97 |
| + 1.6* | 13.81 | 14.8 | 93 |
| 2 Unspiked | 2.79 | - | - |
| + 0.15* | 3.57 | 3.57 | 100 |
| + 0.4* | 5.90 | 5.66 | 104 |
| + 1.6* | 15.47 | 15.66 | 99 |
| 3 Unspiked | 3.70 | - | - |
| + 0.15* | 4.29 | 4.3 | 100 |
| + 0.4* | 5.942 | 6.4 | 93 |
| + 1.6* | 16.49 | 16.4 | 101 |

* - Amount added (ng/0.1 mL)

## LINEARITY

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

| Sample | Obs. Result | Recovery % |
|---|---|---|
| 1 | 3.52 | - |
| 1:2 | 150 | 86 |
| 1:4 | 0.88 | 101 |
| 1:8 | 0.46 | 107 |
| 2 | 5.47 | - |
| 1:2 | 2.7 | 99 |
| 1:4 | 1.21 | 89 |
| 1:8 | 0.66 | 97 |
| 3 | 18.29 | - |
| 1:2 | 8.75 | 96 |
| 1:4 | 4.54 | 99 |
| 1:8 | 2.27 | 100 |

## EXPECTED VALUES

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

| Group | Range (ng/mL) |
|---|---|
| <u>Newborn</u> | |
| 5-30 day | <0.7-2.5 |
| 31-60 day male | 0.8-5.0 |
| 31-60 day female | 0.5-2.3 |
| <u>Children</u> | |
| 3-14 years | 0.07-1.7 |
| <u>Females</u> | |
| Follicular phase | 0.2-1.3 |
| Luteal phase | 1.0-4.5 |

| | |
|---|---|
| Postmenopausal | 0.2-0.9 |

**REFERENCES**


1. Yeo KH, et al. An Automated Solid-Phase 17α-Hydroxyprogesterone 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, Corticosterone, 11- Deoxycorticosterone, Progesterone, 17-hydroxyprogesterone, Cortisol, and Cortisone During Infancy and Childhood. Pediatr Res. 1980; 14(1):39–46.
4. Thorneycroft IH, et al. The Relation of Serum 17-Hydroxyprogesterone and Estradiol 17-beta Levels During the Human Menstrual Cycle. Am J Obstet Gynecol. 1971; 111:947–51.


## Warranty Information

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*For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.*