# Human 17-OH Progesterone ELISA Kit

Enzyme Immunoassay for the quantification of 17-OH Progesterone in Human serum and plasma (EDTA).

Catalog number: ARG82796

Package: 96 wells

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## INTRODUCTION

17α-Hydroxyprogesterone (17α-OHP), also known as 17-OH progesterone[1] (17-OHP), or hydroxyprogesterone (OHP), is an endogenous progestogen steroid hormone related to progesterone. It is also a chemical intermediate in the biosynthesis of many other endogenous steroids, including androgens, estrogens, glucocorticoids, and mineralocorticoids, as well as neurosteroids.

17α-OHP is an agonist of the progesterone receptor (PR) similarly to progesterone, albeit weakly in comparison. In addition, it is an antagonist of the mineralocorticoid receptor (MR) as well as a partial agonist of the glucocorticoid receptor (GR), albeit with very low potency (EC50 >100-fold less relative to cortisol) at the latter site, also similarly to progesterone. [Provided by Wikipedia: 17-OH Progesterone]

## PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. A highly specific antibody for 17-OH progesterone has been pre-coated onto a microplate. 17-OH Progesterone containing samples or standards and a 17-OH Progesterone-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and free 17-OH Progesterone compete for the antibody binding sites. After incubation at room temperature, the wells are washed with diluted Wash Buffer to remove unbound material. Then TMB substrate is added to the wells and color develops in inversely proportion to the amount of 17-OH Progesterone present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of 17-OH Progesterone in the samples is then determined by comparing the O.D of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| Antibody Coated microplate | 8 X 12 strips | 4°C |
| Standards A (0 ng/mL) | 1 mL (ready to use) | 4°C |
| Standards B to F (0.1, 0.4, 1.6, 6.5, 25 ng/mL) | 0.5 mL each (ready to use) | 4°C |
| Control 1 & 2 | 0.5 mL (ready to use) | 4°C |
| 17-OH Progesterone-HRP Conjugate | 11 mL (ready to use) | 4°C |
| 10X Wash Buffer | 50 mL | 4°C |
| TMB substrate | 22 mL (ready to use) | 4°C (protect from light) |
| STOP solution | 7 mL (ready to use) | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Microplate shaker
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

## TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (18-25°C).
- Remove the number of strips required and return unused strips to the pack and reseal.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.

- Run both standards and samples in at least duplicates (triplicate is recommended).

## SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Plasma: Collect blood with EDTA and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Samples containing sodium azide should not be used in the assay.
4. Specimens should be capped and may be stored for up to 7 days at 2-8°C prior to assaying. Specimens stored for a longer time (up to 3 months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.
5. If in an initial assay, a specimen is found to contain more analyte than the highest standard, the specimen can be diluted with Standard A.

## REAGENT PREPARATION

- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 mL of 10X Wash Buffer into 450 mL of distilled water to a final volume of 500 mL) The 1X Wash Buffer is stable for up to 12 weeks at room temperature.

## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 18-25°C) before use. Standards and samples should be assayed in duplicates.

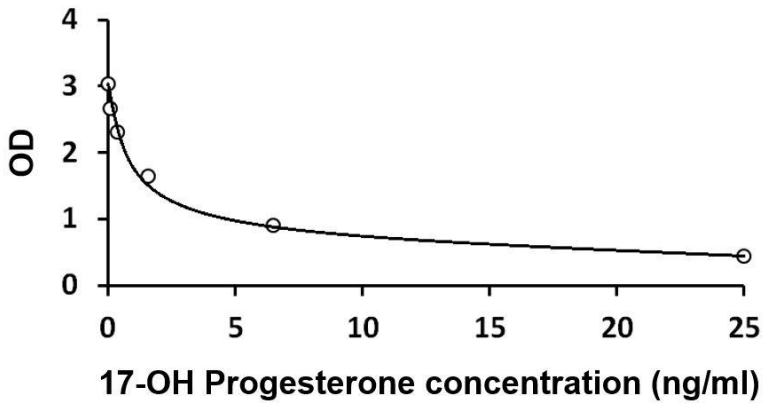
1. Add 25 µL of Standards, Controls and samples into the appropriate wells of the Antibody Coated Microplate.
2. Add 100 µL of 17-OH Progesterone-HRP Conjugate into all wells. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
3. Incubate at RT for 60 minutes on a microplate shaker.
4. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1× Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 200 µL of TMB Substrate to each well, including the blank wells. Incubate in the dark for 30 minutes at RT on a microplate shaker.
6. Immediately Add 50 µL of Stop Solution to each well, including the blank

wells. The color of the solution should change from blue to yellow.

7. Read the OD with a microplate reader at 450 nm immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 15 minutes after adding the stop solution.

## EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human 17-OH Progesterone ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
6. Conversion: Progesterone (ng/ml) X 3.03 = nmol/L

## QUALITY ASSURANCE

### Sensitivity

0.014 ng/mL.

### Specificity

| Substance | Cross Reactivity (%) |
|---|---|
| Androstendione | < 0.1 |
| Testosterone | < 0.1 |
| Cortisol | 0.05 |
| 11-Desoxycortisol | 2.71 |
| Cortisone | 0.19 |
| Corticosterone | < 0.1 |
| 11-Deoxycorticosterone | 0.26 |
| Progesterone | 2.9 |
| Estradiol | < 0.1 |
| Estriol | < 0.1 |
| Estrone | < 0.1 |
| Pregnenolone | 0.17 |
| Prednisolone | < 0.1 |
| Prednisone | 0.11 |
| DHEA | < 0.1 |
| DHEA-S | < 0.1 |
| Danazol | < 0.1 |
| Dexamethasone | < 0.1 |

### Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6.6-8.9% and CV value of inter-assay precision was 6.4-14.9%.

### Recovery

75-122%