Human 17-OH Progesterone (free) ELISA Kit

Enzyme Immunoassay for the quantitative determination of human active free 17-hydroxyprogesterone in saliva

Catalog number: ARG80856

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

17 alpha-Hydroxyprogesterone (17OHP) is a 21-carbon steroid produced by adrenals, ovaries, testes and placenta. 17OHP is the metabolite of progesterone and 17-hydroxyprognenolone, and is a precursor to cortisol. Serum levels are primarily used to diagnose congenital adrenal hyperplasia (CAH). Deficiency of 21-hydroxylase blocks synthesis of cortisol, resulting in an increase of serum 17OHP levels, which leads to increase in ACTH secretion. Besides the classical CAH, late-onset and milder heterozygous forms of CAH also occur. They may clinically manifest themselves during the peripubertal period in the form of menstrual disturbances and hirsutism. Even in adulthood, hirsutism can signal the possible presence of a heterozygous form of CAH. Measurements of basal and post-ACTH stimulation 17OHP levels are helpful in the differential diagnosis of these conditions. The measurement of 17OHP in serum or plasma samples from babies or young children is difficult because the high concentrations of conjugates present in blood. Therefore such serum and plasma samples should be extracted prior to the assay. The importance of the extraction step increases with decreasing age of the patient. In saliva the interference with the conjugates is not relevant at all, as conjugates are not present in saliva. Therefore salivary measurements of 17OHP even in newborns can be done without extraction step! This is not possible in serum or plasma samples.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A highly

specific 17α-OHP antibody has been pre-coated onto a microtiter plate. 17α-OHP containing samples or standards and a 17α-OHP-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and free 17α-OHP compete for the antibody binding sites. After incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of 17α-OHP is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-17α-OHP Conjugate	26 ml (ready to use)	4°C
Standards 0-5 (0, 10, 50, 250, 500, 1000 pg/ml)	6 X 1 ml (ready to use)	4°C
Control Low	1 ml (ready to use)	4°C
Control High	1 ml (ready to use)	4°C
40X Wash Buffer	30 ml	4°C
TMB substrate	25 ml	4°C (Protect from light)
STOP solution	14 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-17α-OHP conjugate before use.
- If crystals are observed in the 40X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The saliva samples should be completely colorless. Even the slightest red color shows blood contamination. Such blood contamination will give falsely elevated concentration values. In case of visible blood contamination the patient should discard the sample, rinse the sampling device with water, wait

for 10 minutes and take a new sample.

Collection –

1. For the correct collection of saliva we are recommending to only use appropriate devices made from ultrapure polypropylene. Do not use any PE devices or Salivates for sampling; this in most cases will result in significant interferences. Glass tubes can be used as well, but in this case special attention is necessary for excluding any interference caused by the stopper.
2. As the steroid secretion in saliva as well in serum shows an obvious episodic secretion pattern it is important to care for a proper timing of the sampling. In order to avoid arbitrary results we are recommending to always taking 5 samples within a period of 2 – 3 hours (multiple sampling) preferably before a meal. As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting. If fasting should be a problem the collection period should be timed just before lunch or before dinner.

Storage and Preparation-

Saliva samples in general are stable at ambient temperature for several days. Therefore mailing of such samples by ordinary mail without cooling will not create a problem. Storage at 4°C can be done for a period of up to one week. Whenever possible samples preferable should be kept at a temperature of -20°C. Even repeated thawing and freezing is no problem. Each sample has to be frozen, thawed, and centrifuged at least once anyhow in order to separate the mucins by centrifugation. Upon arrival of the samples in the lab the

samples have to stay in the deep freeze at least overnight. Next morning the frozen samples are warmed up to room temperature and mixed carefully. Then the samples have to be centrifuged for 5 to 10 minutes. Now the clear colorless supernatant is easy to pipette. If the sample should show even a slightly reddish color it should be discarded. Otherwise the value most probably will be falsely elevated. Due to the episodic variation of steroid secretion we highly recommend the strategy of multiple sampling. If such a set of multiple samples have to be tested the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the aliquots of the 5 single samples in a separate sampling device and perform the testing from this mixture.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- Sample: If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Standard 0 and re-assayed as described in Assay Procedure.

  For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

  a) Dilution 1:10: 10 µl saliva + 90 µl Standard 0 (mix thoroughly)

  b) Dilution 1:100: 10 µl of dilution a + 90 µl Standard 0 (mix thoroughly).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use.

Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 25 µl Standard, controls and samples in duplicate into the appropriate wells.
3. Add 250 µl HRP-17α-OHP antibody into each well. Incubate for 60 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X Wash Buffer (350 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 mixture to each well. Incubate for 15 minutes at room temperature in dark.
6. Add 100 µl of Stop Solution to each well.
7. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

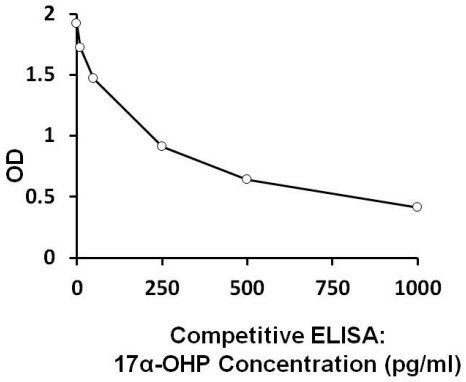
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. 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. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard

deviations of twenty (20) replicate analyses of Standard 0.

Analytical sensitivity is: 2.5 pg/ml

Functional sensitivity of the assay is: 3.6 pg/ml

Specificity

The following substances were tested for cross reactivity of the assay:

Steroid	Cross Reaction (%)
17- α -OH Progesterone	100
Estriol	< 0.01
Estradiol 17β	< 0.01
Testosterone	< 0.01
Dihydrotestosterone	< 0.01
DOC	0.05
11-Desoxycortisol	1.40
Progesterone	1.20
DHEA	< 0.01
DHEA-S	< 0.001
Cortisol	< 0.01
Corticosterone	< 0.05
Aldosterone	< 0.01
Androstenedione	< 0.01
Dehydroepiandrosten sulfate	< 0.01
Prednisone	< 0.01

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 5.23% and the CV value of inter-assay precision was 5.33%.

Recovery

80-113.2%