17-OH-Progesterone ELISA Kit

Catalog Number KA1896

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

An enzyme immunoassay for the quantitative measurement of active free 17-hydroxyprogesterone in saliva.

Principle of the Assay

The 17-OH-Progesterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The microtiter wells are coated with a polyclonal antibody (rabbit) directed towards an antigenic site on the 17α-OHP molecule. Endogenous 17α-OHP of a sample competes with a 17α-OHP-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase conjugate is inversely proportional to the concentration of 17α-OHP in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of 17α-OHP in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Microtiterwells: Wells coated with a anti-cortisol antibody (polyclonal).	96 wells (12x8 (break apart) strips)
Enzyme conjugate: 17α-OHP conjugated to horseradish peroxidase, contain *0.03 % Proclin 300, 0.015% BND and 0.010% MIT as preservative. Ready to use.	26 mL
Substrate Solution: Tetramethylbenzidine (TMB). Ready to use. Hazards identification:  H360 May damage the unborn child.	25 mL
Stopping Solution: Ready to use; containing 0.5 M H_2SO_4. Avoid contact with the stop solution. It may cause skin irritations and burns. Hazards identification:  H290 May be corrosive to metals. H314 Causes severe skin burns and eye damage.	14 mL
Wash Solution (40X concentrated)	30 mL

Standards and Controls - Ready to use

Component	Concentration (pg/mL)	Amount
Standard A (0)	0	1 mL
Standard B (1)	10	1 mL
Standard C (2)	50	1 mL
Standard D (3)	250	1 mL
Standard E (4)	500	1 mL
Standard F (5)	1000	1 mL
Control 1 (Control low)	Refer to vial labels or QC-Datasheet!	1 mL
Control 2 (Control high)		1 mL

Conversion: pg/mL x 3.03 = pmol/L

*0.03 % Proclin 300, 0.015% BND and 0.010% MIT as preservative.

Note: Additional Standard A for sample dilution is available upon request.

*BND = 5-bromo-5-nitro-1,3-dioxan

MIT = 2-methyl-2H-isothiazol-3-one

Storage Instruction

When stored at 2° to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2° to 8°C. Microplate wells must be stored at 2° to 8°C. Once the foli bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for six weeks if stored as described above.

Materials Required but Not Supplied

- ✓ A microtiter plate calibrated reader (450±10 nm)
- ✓ Calibrated variable precision micropipettes (25 µL, 100 µL, 200 µL, 250 µL).
- ✓ Absorbent paper.
- ✓ Distilled or deionized water
- ✓ Timer (60 min. range)
- ✓ Semi-logarithmic graph paper or software for data reduction

Precautions for Use

1. This kit is for research use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2-8°C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature WILL affect the absorbance readings of the assay. However, Values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink, or apply cosmetics in areas where specimens or kit reagents are handled.

12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use reagents from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristic of the plates may result slightly different.
17. Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin, BND and MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. The disposal of the kit must be made according to the national regulations.
22. For information on hazardous substances included in the kit please refer to Material Safety Data Sheet (MSDS). Material Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
23. Damaged tested kits: In case of any severe damage of the kit or components, the manufacture have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be store until a final solution has been found. After this, they should be disposed according to the official regulations.
24. Disposal of the Kit: The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

Assay Protocol

Reagent Preparation

Bring all reagents and required number of strips to room temperature before use.

- Wash Solution

  Add deionized water to the 40x concentrated wash solution.

  Dilute 30 mL of concentrated wash solution with 1170 mL deionized water to a final volume of 1200 mL.

  The diluted wash solution is stable for 2 weeks at room temperature.

Sample Preparation

✓ Sample collection and preparation

Eating, drinking, chewing gums or brushing teeth should be avoided for 30 minutes before sampling.

Otherwise, it is recommended to rinse mouth thoroughly with cold water 5 minutes prior to sampling.

Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

In case of visible blood contamination discard the sample, rinse the sampling device with water, wait for 10 minutes and take a new sample.

Note: Samples containing sodium azide should not be used in the assay.

✓ Sample Collection

It is recommended to collect saliva samples with commercially available equipment.

Do not use any cotton swab for sampling, such as Salivettes; this in most cases will result in significant interferences.

Due to the episodic secretion pattern of steroid hormones it is important to care for a proper timing of the sampling.

In order to avoid arbitrary results we recommend that always 5 samples be taken 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.

✓ Specimen Storage and Preparation

Specimens should be capped and may be stored for up to one week at 4°C prior to assaying.

Specimens held for a longer time should be frozen -20°C prior to assay. 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 (at 3000 - 2000 x g).
Now the clear colorless supernatant is easy to pipette.
If 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.

✓ Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Standard A 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 A (mix thoroughly)
b) Dilution 1:100: 10 µL of dilution a) + 90 µL Standard A (mix thoroughly).

Assay Procedure

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Each run must include a standard curve.
All standards, samples, and controls should be run in duplicate. All standards, samples, and controls should be run concurrently so that all conditions of testing are the same.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense 25 µL of each 17α-OHP Standard, Control and samples with new disposable tips into appropriate wells.
3. Dispense 250 µL Enzyme Conjugate into each well.
   Mix the plate thoroughly for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for 60 minutes at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note: The sensitivity and precision of this assay is markedly influenced by the correct

performance of the washing procedure!

6. Add 200 µL of Substrate Solution to each well.
7. Incubate for 15 minutes at room temperature.
8. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well.
9. Determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader.
   It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

Data Analysis

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using semi-logarithmic 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.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 1000 pg/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

✓ Example of typical calibration curve

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

Standard	Optical Units (450nm)
Standard A (0 pg/mL)	1.92
Standard B (10 pg/mL)	1.72
Standard C (50 pg/mL)	1.47
Standard D (250 pg/mL)	0.91
Standard E (500 pg/mL)	0.64
Standard F (1000 pg/mL)	0.41

Performance Characteristics

- Expected Normal Values

  In order to determine the normal range of SLV 17α-OHP, two studies were performed using saliva samples from 129 apparently healthy children ages 6 - 12 years, 132 males ages 21 to 70 years, and 252 females of non-pregnant women with regular menstrual cycles, ages 21 to 50 years. Saliva samples were collected in the morning, frozen at -20°C, and analyzed using the17-OH-Progesterone ELISA Kit. The following ranges were calculated from this study.

Summary Normal ranges for 17-OH-Progesterone ELISA Kit

	Age group		Mean	S.D.	Range 5 - 95%
Children	6 - 12 yrs	N = 129	16.9 pg/mL	9.5 pg/mL	3.0 - 32.9 pg/mL
Women	21 - 50 yrs	Follicular phase: N = 124	22.0 pg/mL	11.1 pg/mL	8.2 - 41.1 pg/mL
		Luteal phase: N = 128	51.2 pg/mL	17.3 pg/mL	28.1 - 84.8 pg/mL
Men	21 - 70 yrs	N = 152	24.9 pg/mL	12.6 pg/mL	10.6 - 54.8 pg/mL

We recommend that each laboratory determine its own range for the population tested.

- Quality Control

  Good laboratory practice requires that controls be run with each standard curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

  It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and abnormal levels.

  The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

  It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

  Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid.

  In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

  After checking the above mentioned items without finding any error contact your distributor or the manufacturer directly.

- Sensitivity

  The analytical sensitivity of the ELISA was calculated by subtracting 2 standard deviation from the mean of 20 replicate analyses of the zero Standard (Standard A).

  Analytical sensitivity is: 2.5 pg/mL

  Functional sensitivity of the assay is: 3.6 pg/mL

- Specificity of Antibodies (Cross Reactivity)

  The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to 17α-OHP.

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

- Assay Dynamic Range

  The range of the assay is between 3.6 - 1000 pg/mL.

- Precision

✓ Intra-Assay

The intra-assay reproducibility was determined by replicate measurements of 6 saliva samples using ELISA Kit. The within assay precision is shown below:

	sample 4	sample 3	sample 1	sample 2	Sample 6	sample 5
Mean (pg/mL)	24.2	37.5	63.3	90.3	447.4	820.4
S.D.	1.9	1.4	4.6	4.5	17.8	29.0
CV (%)	8.0	3.6	7.3	5.0	4.0	3.5
n =	20	20	20	20	20	20

✓ Inter-Assay

The inter-assay variation was determined by duplicate measurements of six saliva samples on 10 different days using 17-OH-Progesterone ELISA Kit. Mean of the duplicate measurements is given below.

	sample 4	sample 1	sample 5	sample 2	sample 3	Sample 6
Mean (pg/mL)	9.4	50.7	101.1	211.8	512.5	800.2
SD	1.0	1.7	7.6	8.3	10.3	38.2
CV (%)	10.4	3.4	7.5	3.9	2.0	4.8
n =	20	20	20	20	20	20

✓ Inter-Lot

The inter-assay (between-lots) variation was determined by repeated measurements of six saliva samples in three different kit lots.

	sample 4	sample 1	sample 5	sample 2	sample 3	sample 6
Mean pg/mL	10.2	44.3	100.4	205.3	515.7	798.1
SD pg/mL	1.3	1.5	6.1	3.6	19.5	46.8
CV (%)	12.4	3.4	6.1	1.8	3.8	5.9
n =	9	9	9	9	9	9

- Recovery

Recovery of this ELISA was determined by adding increasing amounts of the analyte up to 500 pg/mL to three different saliva samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

		saliva 2	saliva 3	saliva 1	saliva 4	saliva 5	saliva 6
Concentration pg/mL		3.7	17.9	282.8	824.1	1200.0	1050.0
Average % Recovery		104.1	102.1	101.7	98.3	100.4	99.3
Range of % Recovery	from	97.7	96.6	98.2	93.1	94.5	93.7
	to	103.9	107.5	109.5	103.8	105.2	103.5

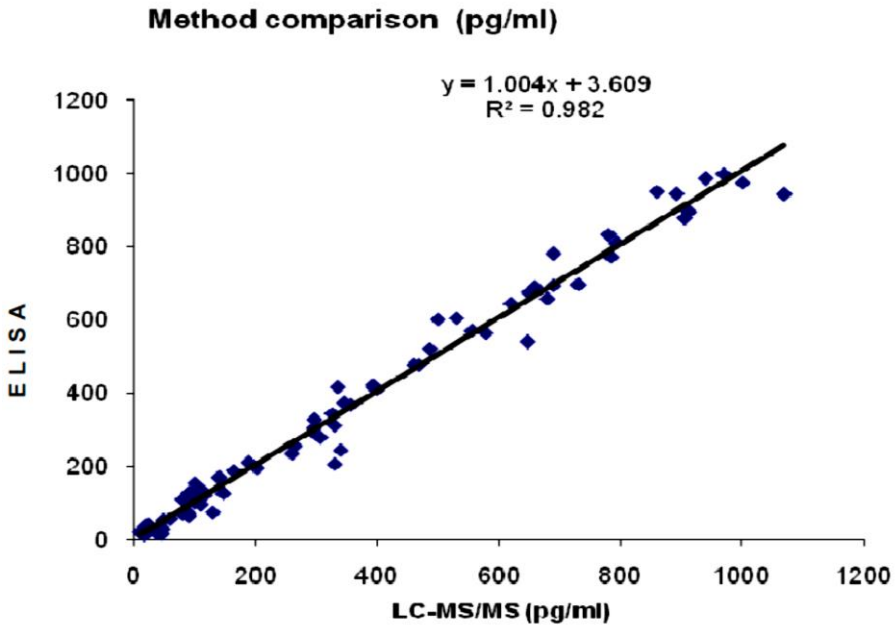
- Linearity

Six saliva samples containing different amounts of analyte (spiked and unspiked) were serially diluted with zero standard and assayed with this ELISA. Three native samples were serially diluted up to 1:128, and 3 samples were spiked with 17α-OHP and then serially diluted up to 1:10. The percentage recovery was calculated by comparing the expected and measured values for 17α-OHP. An assay linearity of 3.6 - 1000 pg/mL has been identified as the usable range. Samples above this range must be diluted and re-run.

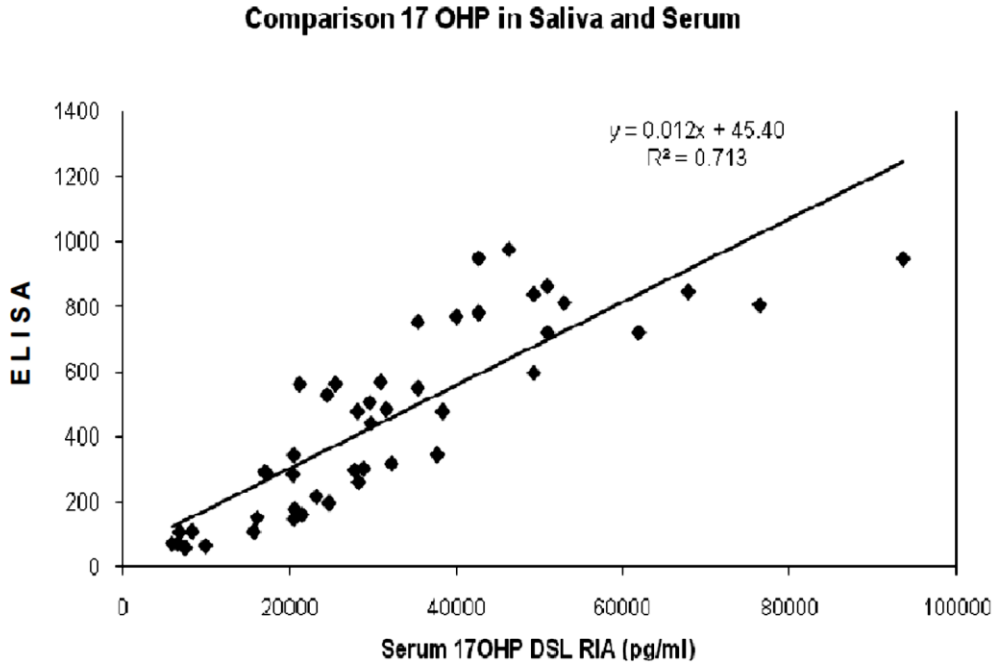
		sample 2	sample 1	sample 3	sample 6	sample 5	sample 4
Concentration pg/mL		39.3	96.6	194.9	816.6	1050.0	1200.0
Average % Recovery		99.3	95.0	100.5	97.6	99.3	100.4
Range of % Recovery	from	87.2	86.1	91.5	93.8	93.7	80.0
	to	107.4	99.0	113.2	103.5	103.5	105.2

- Comparison Studies

A study was performed using non spiked Saliva from adult normal and CAH-persons were assayed in parallel with this ELISA Kit and a reference LC-MS. Seventy-six saliva samples were tested on both the LC-MS and the SLV ELISA test. The following results were obtained.



Another study was performed using 60 serum and saliva samples were taken in parallel at 8:00 am and assayed using the Salivary 17-OH ELISA for the saliva samples and a Coated Tube RIA for 17-OHP for the sera samples. The persons were a collective of 22 children and young adults suffering from congenital adrenal hyperplasia (CAH). The results cover several years.



- Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results. The person should not eat, drink, chew gum or brush teeth for 30 minutes before sampling. Otherwise rinse mouth thoroughly with cold water 5 min prior to sample collection. Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

- Interfering Substances

Blood contamination of more than 0.08% in saliva samples will affect results, and usually can be seen by eye. Therefore, samples containing any visible blood should not be used.
Concentrations of Sodium Azide > 0.02% interferes in this assay and may lead to false results.

- High-Dose-Hook Effect

No hook effect was observed in this test.

Resources

Reference

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Plate Layout

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