Product information

User´s Manual

17α-Hydroxy-progesterone (17α-OHP) free in Saliva ELISA

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

For research use only, not for use in diagnostic procedures.

REF **IB79302**

Σ **96**

Manufactured for:
Immuno-Biological Laboratories, Inc. (IBL-America)
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1 INTRODUCTION

1.1 Intended Use

An enzyme immunoassay for the detection of active free 17-hydroxyprogesterone in saliva. For research use only, not for use in diagnostic procedures.

2 PRINCIPLE OF THE TEST

The IBL-America Salivary 17α-OHP 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 an unknown 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 unknown. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of 17α-OHP in the unknown.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only. For professional 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 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of unknowns 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 unknowns will not be affected.
10. Never pipet by mouth and avoid contact of reagents and biologicals with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where biologicals or kit reagents are handled.
12. Wear disposable latex gloves when handling unknowns and reagents. Microbial contamination of reagents or biologicals 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 components 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 characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 1 N acidic solution. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or 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. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from IBL AMERICA.

4 REAGENTS

4.1 Reagents provided

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with a anti-17α-OHP antibody (polyclonal).
2. ***Calibrator (Calibrator 0-5)*,** 6 vials, 1 mL, ready to use;
   Concentrations: 0 – 10 – 50 – 250 – 500 – 1000 pg/mL
   Conversion: pg/mL x 3.03 = pmol/L
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative.
3. ***Control Low & High***, 2 vials, 1.0 mL each, ready to use;
   Control values and ranges please refer to vial label or QC-Datasheet.
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative.
4. ***Enzyme Conjugate***, 1 vial, 26 mL, ready to use;
   17α-OHP conjugated to horseradish Peroxidase;
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative.
5. ***Substrate Solution***, 1 vial, 25 mL, ready to use;
   Tetramethylbenzidine (TMB).
6. ***Stop Solution***, 1 vial, 14 mL, ready to use;
   contains 0.5M H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
7. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see „Preparation of Reagents“.

* BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

Note: Additional *Calibrator 0* for dilution of unknowns is available upon request.

4.2 Material required but not provided

- 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

4.3 Storage Conditions

When stored at 2 °C 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 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil 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.

4.4 Reagent Preparation

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

4.5 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.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, IBL AMERICA has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 COLLECTION AND HANDLING OF UNKNOWNS

Unknowns containing sodium azide should not be used in the assay.
The saliva unknowns 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 unknown should be discarded, the sampling device should be rinsed with water, and after waiting for 10 minutes, a new unknown should be collected.

5.1 Collection of Unknowns

For the correct collection of saliva we are recommending to only use appropriate devices made from ultra-pure polypropylene. Do not use any PE devices or Salivettes for collection; 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. Please contact IBL-America for more details.

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 collection. In order to avoid arbitrary results we are recommending to always take 5 unknowns within a period of 2 – 3 hours (multiple sampling) preferably before a meal. As food might contain significant amounts of steroid hormones unknowns preferably should be taken while fasting. If fasting should be a problem the collection period should be timed just before lunch or before dinner.

5.2 Storage and Preparation of Unknowns

Saliva unknowns in general are stable at ambient temperature for several days. Therefore mailing of such unknowns 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 unknowns preferable should be kept at a temperature of -20°C. Even repeated thawing and freezing is no problem. Each unknown has to be frozen, thawed, and centrifuged at least once anyhow in order to separate the mucins by centrifugation. Upon arrival of the unknowns in the lab the unknowns have to stay in the deep freeze at least overnight. Next morning the frozen unknowns are warmed up to room temperature and mixed carefully. Then the unknowns have to be centrifuged for 5 to 10 minutes. Now the clear colorless supernatant is easy to pipette. If the unknown should show even a slighty 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 unknowns have to be tested the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the aliquots of the 5 single unknowns in a separate sampling device and perform the testing from this mixture.

5.3 Dilution of Unknowns

If in an initial assay, an unknown is found to contain more than the highest calibrator, the unknowns can be diluted with *Calibrator 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 *Calibrator 0* (mix thoroughly)
b) Dilution 1:100: 10 µl of dilution a + 90 µl *Calibrator 0* (mix thoroughly).

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and unknowns 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 calibrator, control or unknown 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.

6.2 Test Procedure

Each run must include a calibration curve.
All calibrators, unknowns, and controls should be run in duplicate. All calibrators, unknowns, 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 *Calibrator, Control* and unknown 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*.

6.3 Results

1. Calculate the average absorbance values for each set of calibrators, controls and unknowns.
2. Using semi-logarithmic graph paper, construct a calibration curve by plotting the mean absorbance obtained from each calibrator 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 unknown determine the corresponding concentration from the calibration 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 unknowns can be read directly from this calibration curve. Unknowns with concentrations higher than that of the highest calibrator 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.

7 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration 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 controls according to state and federal regulations. The use of controls is advised to assure the day to day validity of 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 unknowns being tested 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 IBL AMERICA directly.

8 PERFORMANCE CHARACTERISTICS

8.1 Assay Dynamic Range

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

8.2 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

8.3 Recovery

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

		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	**from**	97.7	96.6	98.2	93.1	94.5	93.7
% Recovery	**to**	103.9	107.5	109.5	103.8	105.2	103.5

8.4 Linearity

Six saliva unknowns containing different amounts of analyte (spiked and unspiked) were serially diluted with zero calibrator and assayed with the IBL-America ELISA. Three native unknowns were serially diluted up to 1:128, and 3 unknowns 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. Unknowns above this range must be diluted and re-run.

		Unknown 2	Unknown 1	Unknown 3	Unknown 6	Unknown 5	Unknown 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	**from**	87.2	86.1	91.5	93.8	93.7	80.0
% Recovery	**to**	107.4	99.0	113.2	103.5	103.5	105.2

9 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 unknowns or modification of this test might influence the results.

The subject 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 collection of unknowns. Do not collect unknowns when oral diseases, inflammation or lesions exist (blood contamination).

9.1 Interfering Substances

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

9.2 High-Dose-Hook Effect

No hook effect was observed in this test.

10 LEGAL ASPECTS

1. Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IBL AMERICA.

2. Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 10.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

11 ORDERING INFORMATION

This kit is manufactured for Immuno-Biological Laboratories, Inc. (IBL-America). For ordering information, please contact:

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SYMBOLS USED WITH IBL-AMERICA ELISAS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consultez le Mode d'emploi	Consulte las Instrucciones	Consulti le istruzioni
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Diagnostic in vitro	Diagnóstico in vitro	Diagnostica in vitro
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Contenu	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
Microtiterwells	Microtiterwells	Mikrotiterwells	Plaques de micro-titration	Placas multipocillo	Micropozzetti
Antiserum	Antiserum	Antiserum	Antisérum	Antisuero	Antisiero
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
Enzyme Complex	Enzyme Complex	Enzymkomplex	Complexe enzymatique	Complex enzimático	Complesso enzimatico
Substrate Solution	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
Stop Solution	Stop Solution	Stopplösung	Solution d'arrêt	Solución de parada	Soluzione d´ arresto
Zero Calibrator	Zero Calibrator	Nullstandard	Standard 0	Estándar 0	Standard zero
Calibrator	Calibrator	Standard	Standard	Estándar	Standard
Control	Control	Kontrolle	Contrôle	Control	Controllo
Assay Buffer	Assay Buffer	Assaypuffer	Tampon d'essai	Tampón de ensayo	Tampone del test
Wash Solution	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH (idrossido di sodio 1N)
1 N HCl	1 N HCl	1 N HCl	1N HCl	1 N HCl	
Sample Diluent	Sample Diluent	Probenverdünnungs-medium	Solution pour dilution de l'échantillon	Solución para dilución de la muestra	Diluente dei campioni
Conjugate Diluent	Conjugate Diluent	Konjugatverdünnungs-medium	Solution pour dilution du conjugué	Solución para dilución del conjugado	Diluente del tracciante

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