Instructions for Use

17-OH-Progesterone ELISA

Enzyme immunoassay for the in-vitro-diagnostic quantitative determination of 17-OH-Progesterone in human serum and plasma.

REF **RE52071**

Σ **96**

2-8°C

EU: IVD CE

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1. INTENDED USE

Enzyme immunoassay for the *in-vitro diagnostic* quantitative determination of 17-OH-progesterone in human serum and plasma.

2. SUMMARY AND EXPLANATION

The steroide hormone 17-OH-progesterone (17-OHP) is produced in the adrenal cortex and in the gonads. Gestagenic effects exerted by 17-OHP are only small. Nevertheless, this hormone is of clinical significance because it represents the ultimate precursor of 11β-desoxycortisol (compounds, CpS). CpS is formed by hydroxylation of the carbon atom C 21. Enzyme activity of 21-hydroxylase in the adrenal cortex may thus be monitored by analyzing the level of 17-OHP in the blood.
Deficiencies in 21-hydroxylase, most commonly found in congenital adrenal hyperplasia, result in excessive secretion of 17-OHP and consequently in enhanced blood levels. Deficiencies in 11-hydroxlase, however, merely lead to moderately increased values of 17-OHP. The analysis of this steroid hormone, therefore, plays a significant role in the differential diagnosis of congenital adrenal hyperplasia.
In adult non-pregnant women, 17-OHP levels in the blood depend on the phase of the menstrual cycle. Like progesterone, 17-OHP is secreted by the mature follicle and the corpus luteum. Concentrations are generally higher after ovulation.
In addition, levels of 17-OHP are influenced by daytime rhythms which correlate with the adrenal secretion of cortisol. Maximal levels are found in samples collected between midnight and 8.00 a.m..
In adult men, there are few indications of similar fluctuations of 17-OHP levels.
During pregnancy, large amounts of 17-OHP are produced by the fetus, the placenta and the adrenal cortex. The hormone is secreted into the fetal and the maternal blood circulation. Maternal values of 17-OHP strongly increase after the 32. week of pregnancy reaching 4-fold higher levels than during the luteal phase of the menstrual cycle. 17-OHP may also be found in the umbilical cord of newborns.

3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed colour is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. 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.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution. It may cause skin irritations and burns.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the indicated expiry after the kit is broken. Make sure that the broken bag is tightly closed when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma (EDTA)

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sunlight.
Stability:	7 days	3 months	Avoid repeated freeze-thaw cycles.

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal), 17-OH-Progesterone Antiserum.
1 x 20 mL	ENZCONJ	**Enzyme Conjugate** Yellow Colored. Ready to use. Contains: 17-OH-Progesterone, conjugated to HRP, phosphate buffer, stabilizers.
1 x 2.0 mL 6 x 1.0 mL	CAL A-G	**Standard A-G** 0; 0.15; 0.5; 1.5; 3.0; 7.5; 20 ng/mL 0.45; 1.5; 4.5; 9.1; 22.7; 60.6 nmol/L Ready to use. Standard A = Zero Standard (steroid-free serum). Contains: 17-OH-Progesterone, Human serum, stabilizers.
2 x 1.0 mL	CONTROL 1+2	**Control 1+2** Ready to use. Concentrations / acceptable ranges see QC certificate.
1 x 50 mL	WASHBUF CONC	**Wash Buffer** Concentrate (20x) Contains: phosphate buffer, Tween, stabilizers.
1 x 15 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB, Buffer, stabilizers.
1 x 15 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1 M H_2SO_4.
3 x	FOIL	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 5; 25; 50; 100; 500 µL
2. Vortex mixer
3. 8-Channel Micropipettor with reagent reservoirs
4. Wash bottle, automated or semi-automated microtiter plate washing system
5. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
6. Bidistilled or deionised water
7. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of lyophilized or concentrated components

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
15 mL	**WASHBUF CONC**	ad 300 mL	bidist. water	1:20	Mix vigorously.	2-8°C	4 weeks

10.2. Dilution of Samples

Samples suspected to contain concentrations higher than the highest standard have to be diluted with Standard A.

11. TEST PROCEDURE

1.	Pipette **25 µL** of each **Standard, Control and sample** into the respective wells of the Microtiter Plate.
2.	Pipette **200 µL** of **Enzyme Conjugate** into each well.
3.	Cover plate with adhesive foil. Shake plate carefully. **Incubate 60 min** at **RT (18-25°C).**
4.	Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
6.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
7.	**Incubate 30 min** at **RT (18-25°C).**
8.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
9.	**Measure** optical density with a photometer at **450 ± 10 nm** (Reference-wavelength: 600-650 nm) within **30 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

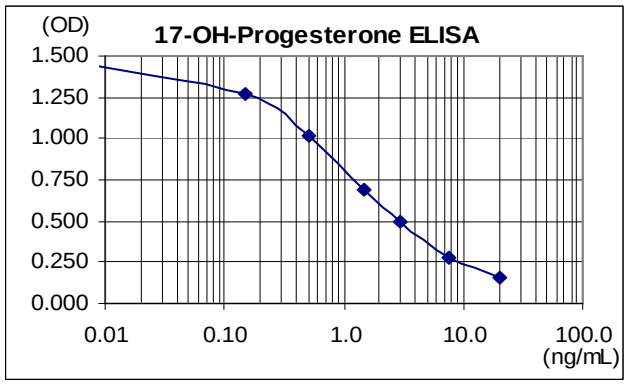
Conversion:

17-OH-Progesterone (ng/mL) x 3.03 = nmol/L

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	17-OH-Progesterone (ng/mL)	OD_{Mean}	OD/OD_{max} (%)
A	0.00	1.487	100.00
B	0.15	1.276	85.80
C	0.50	1.015	68.30
D	1.50	0.691	46.50
E	3.00	0.496	33.30
F	7.50	0.274	18.40
G	20.00	0.152	10.20



14. EXPECTED VALUES

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

Apparently healthy subjects show the following values:

17-OH-Progesterone (ng/mL)						
	Females			Males		
Phase	**Mean**	**Range**	**N**	**Mean**	**Range**	**N**
Follicular Phase	0.51	0.3 – 1.0	16	0.75	0.05 - 1.6	48
Luteal Phase	0.96	0.2 - 2.9	16			
after ACTH Stimulation	1.4	< 3	17			
Pregnancy (3rd trimester)	9.1	1.8 – 20.0	52			

It is recommended that each laboratory establishes its own range of normal values.

15. LIMITATIONS OF THE PROCEDURE

The following blood components do not have a significant effect (+/-20% of expected) on the test results up to the below stated concentrations:

Hemoglobin	8.33 mg/mL
Bilirubin	0.33 mg/mL
Triglyceride	0.25 mg/mL

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)		Cross-reactivity of other substances tested ≤ 0.001 %
	17α-OH-Pregnenolone	1.7		
	Progesterone	1.4		
	11-Desoxy-Cortisol	1.3		
	Desoxy-Corticosterone	0.12		
	Cortisol	0.013		
	Pregnenolone	0.012		
Analytical Sensitivity (Limit of Detection)	0.03 ng/mL	Mean signal (Zero-Standard) - 2SD		
Precision	Range (ng/mL)	CV (%)		
Intra-Assay	2.44 – 11.41	2.8 – 4.9		
Inter-Assay	0.26 – 5.74	5.8 – 9.2		
Linearity	Range (ng/mL)	Serial dilution up to	Range (%)	
	1.56 – 9.23	1:16	80 - 120	
Recovery	Mean (%)	Range (%)	% Recovery after spiking	
	101	80 - 121		
Method Comparison versus GCMS	IBL-Assay = 1.31 x GCMS – 0.45		r = 0.94; n = 22	

17. PRODUCT LITERATURE REFERENCES

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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