Instructions for Use

Cortisol Saliva ELISA

Enzyme Immunoassay for the quantitative determination of free cortisol in human saliva.

REF **RE52611**

Σ **96**

EU: IVD CE

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1. INTENDED USE

Enzyme immunoassay for the quantitative determination of free Cortisol in human saliva, as an aid in the assessment of Cushing Syndrome and Addison's Disease.

2. SUMMARY AND EXPLANATION

Cortisol (hydrocortisone, compound F) is the main glucocorticoid in humans and is produced in the zona fasciculata of the adrenal cortex. 90 % of the circulating cortisol are bound to corticoid binding globulin (CBG, Transcortin), ca. 7 % are bound to albumin and only 1–3 % are unbound. Only the latter part represents the active form of cortisol.

The free cortisol is released in saliva and is excreted via the kidneys as a small part among the metabolites of cortisol. The level of free cortisol in blood regulates mainly its secretion in the adrenal cortex in a negative feedback mechanism via CRH (corticotropin releasing hormone) in the hypothalamic region and the ACTH in the pituitary gland, but it is also affected by different situations above all by stress.

In humans there is a physiological fluctuation of cortisol achieving the highest level in the morning and the lowest during midnight. This fluctuation of cortisol plasma level is reflected in saliva normally with a peak in the first 90 minutes after wake up.

The cortisol measurement is indicated in diseases with abnormal gluco-corticoid production e.g. Cushing Syndrome and Addison's Disease. Because of the diurnal fluctuation of cortisol levels it is necessary to take several samples for an individual cortisol profile or during dynamic tests like dexamethasone-suppression- or ACTH-stimulation-test. Therefore a salivary sample collection is an easy method without the stress of repeated venipunctures. The measurement of cortisol in saliva is advisable in patients with abnormal CBG levels such as women in pregnancy, people with hypothyroidism, nephrotic syndrome or marked adipositas and during the application of different drugs, including oral contraceptives.

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. Some reagents contain ProClin 300 as preservative. In case of contact with eyes or skin, flush immediately with water. When disposing reagents, flush with a large volume of water to avoid built-up.
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 opened. Make sure that the opened bag is tightly closed when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Saliva

The patient should not eat, drink, chew gums or brush teeth for 30 min 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).

Saliva can be collected in a suitable sampling device. A minimum of 0.5 mL liquid should be collected. Saliva flow can be stimulated by chewing on a piece of Parafilm®. It is recommended to freeze samples at –20°C prior to laboratory testing. After thawing, mix and centrifuge 10 min at 2000 – 3000 x g to remove particulate material.

Take care that the saliva samples are visually acceptable.
(Reddish color indicating blood contamination)

Warning: Salivette® with Citric Acid leads to incorrect results!

Storage:	37°C	18-25°C	2-8°C	≤ -20°C (Aliquots)
Stability:	1 week	> 2 weeks	> 4 weeks	≥ 6 months

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with anti-cortisol antibodies (rabbit).
1 x 13 mL	ENZCONJ	**Enzyme Conjugate** Yellow Colored. Ready to use. Contains: Cortisol (chromatographically purified), conjugated to HRP, stabilizers.
1 x 3.5 mL 5 x 1.0 mL	CAL A-F	**Standard A-F** 0; 0.015; 0.04; 0.17; 0.70; 3.00 µg/dL 0; 0.15; 0.4; 1.7; 7.0; 30 ng/mL 0; 0.41; 1.10; 4.69; 19.3; 82.8 nmol/L Ready to use. Contains: Cortisol, Buffer, < 0.1 % BSA, < 0.1 % ProClin.
2 x 1.0 mL	CONTROL 1+2	**Control 1+2** Ready to use. Contains: Cortisol, low and high, Buffer, < 0.1 % BSA, < 0.1 % ProClin. Exact concentrations see vial labels or QC certficate.
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.
1 x 100 mL	WASHBUF CONC	**Wash Buffer** Concentrate (10x) Contains: phosphate buffer and Tween.
3 x	FOIL	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 5; 20; 50; 100; 1000 µL
2. A suitable sampling device should be used.
3. Orbital shaker (400-600 rpm)
4. Vortex mixer
5. 8-Channel Micropipettor with reagent reservoirs
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
8. Bidistilled or deionised water
9. 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

	The contents of the kit for 96 determinations can be divided into 3 separate runs. **The volumes stated below are for one run with 4 strips (32 determinations).**

Preparation of concentrated components

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
10 mL	**WASHBUF**	ad 100 mL	bidist. water	1:10	Mix vigorously.	2-8°C	8 weeks

11. TEST PROCEDURE

1.	Pipette **50 µL** of each **Standard, Control and sample** into the respective wells of the microtiter plate.
2.	Pipette **100 µL** of **Enzyme Conjugate** into each well. Cover plate with adhesive foil. Shake plate carefully.
3.	**Incubate 2 h** at **RT (18-25°C)** on an orbital shaker (400 – 600 rpm).
4.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **250 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
6.	**Incubate 30 min** at **RT (18-25°C)** on an orbital shaker (400 – 600 rpm).
7.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Shake briefly. Color changes from blue to yellow.
8.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **15 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.

Saliva samples with remarkably elevated values should be reviewed for blood contamination.

Conversion:

Cortisol (ng/mL) x 2.76 = nmol/L

Cortisol (µg/dL) x 27.6 = nmol/L

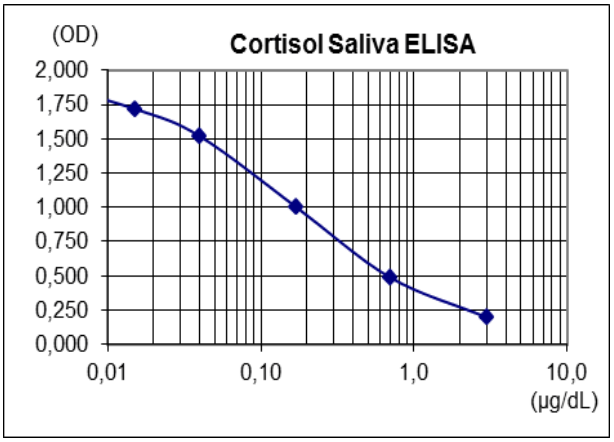
Reportable range:

Saliva: 0.015 – 3 µg/dL Cortisol

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Cortisol (µg/dL)	OD_{Mean}	OD/OD_{max} (%)
A	0.00	1.946	100
B	0.015	1.719	88.3
C	0.04	1.519	78.1
D	0.17	1.003	51.5
E	0.70	0.488	25.1
F	3.00	0.198	10.2



Standards and Controls are calibrated by use of an isotope dilution-LCMS as reference method.

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:

♂/♀ n = 100 Age > 6 y	Cortisol (Saliva) Range					
Time after awakening (h)	Median (µg/dL)	Range (µg/dL) percentile		Median (nmol/L)	Range (nmol/L) percentile	
		5%	95%		5%	95%
awakening	0.343	0.113	0.803	9.47	3.12	22.17
0.5	0.478	0.200	1.076	13.19	5.52	29.70
1	0.384	0.101	0.936	10.60	2.79	25.82
2	0.234	0.083	0.574	6.44	2.29	15.85
5	0.150	0.074	0.355	4.14	2.04	9.79
8	0.116	0.055	0.314	3.20	1.53	8.67
12	0.082	0.032	0.322	2.26	0.87	8.89

♂/♀ n = 112 Age: 18-70 y	Cortisol (Saliva) Range							
	Median (µg/dL)	Range (µg/dL) percentile		Max	Median (nmol/L)	Range (nmol/L) percentile		Max
		5%	95%			5%	95%	
midnight value	0.021	0.006	0.108	0.274	0.58	0.17	2.98	7.56

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

15. LIMITATIONS OF THE PROCEDURE

Children levels have not yet been evaluated with this test.

Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

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

The following substances do not have a significant effect on the test results up to the below stated concentrations (+/- 20%).	Substance	Concentration	Cortisol (µg/dL)
	Blood	0.25 %	0.16; 0.26; 1.09
	NaN_3	0.25 %	0.18; 0.21; 0.33

16. PERFORMANCE

<table>
<tr><td rowspan="9">Analytical Specificity (Cross Reactivity)</td><td colspan="2">Substance</td><td colspan="2">Cross Reactivity (%)</td><td rowspan="9">Cross-reactivity of other substances tested < 0.01 %</td></tr>
<tr><td colspan="2">Prednisolone</td><td colspan="2">30</td></tr>
<tr><td colspan="2">11-Desoxy-Cortisol</td><td colspan="2">7.0</td></tr>
<tr><td colspan="2">Corticosterone</td><td colspan="2">1.4</td></tr>
<tr><td colspan="2">Cortisone</td><td colspan="2">4.2</td></tr>
<tr><td colspan="2">Prednisone</td><td colspan="2">2.5</td></tr>
<tr><td colspan="2">17α-OH-Progesterone</td><td colspan="2">0.4</td></tr>
<tr><td colspan="2">Desoxy-Corticosterone</td><td colspan="2">0.9</td></tr>
<tr><td colspan="2">6α-Methyl-17α-OH-Progesterone</td><td colspan="2">0.04</td></tr>
<tr><td>Analytical Sensitivity (Limit of Detection)</td><td>0.003 µg/dL</td><td colspan="4">Mean signal (Zero-Standard) - 2SD</td></tr>
<tr><td>Functional Sensitivity</td><td>0.005 µg/dL</td><td colspan="4">Mean Conc. < 20 % CV</td></tr>
<tr><td rowspan="3">Precision</td><td></td><td>Range (µg/dL)</td><td colspan="2">CV (%)</td><td>n</td></tr>
<tr><td>Intra-Assay</td><td>0.066 – 1.091</td><td colspan="2">3.2 – 6.1</td><td>20</td></tr>
<tr><td>Inter-Lot</td><td>0.035 – 2.288</td><td colspan="2">4.2 – 17.0</td><td>10</td></tr>
<tr><td rowspan="2">Linearity</td><td colspan="2">Range (µg/dL)</td><td colspan="3">Range (%)</td></tr>
<tr><td colspan="2">0.013 – 2.208</td><td colspan="3">82.1 – 102.0</td></tr>
<tr><td rowspan="2">Recovery (n=4)</td><td>Mean (%)</td><td>Range (%)</td><td colspan="3" rowspan="2">% Recovery after spiking
(Added concentration 0.02 – 2.5 µg/dL)</td></tr>
<tr><td>105.3</td><td>98.0 – 117.9</td></tr>
<tr><td>Method Comparison</td><td colspan="4">IBL-ELISA = 0.970 x Luminescence + 0.008
IBL-ELISA = 1.015 x LCMS - 0.028</td><td>r = 0.99; n = 48
r = 0.99; n = 58</td></tr>
</table>

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.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.


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