

Instructions for Use

**IBL**

# Cortisol ELISA

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

**REF** **RE52061**

 **96**

   **2-8°C**

EU: **IVD**  U.S.: *For research use only.  
Not for use in diagnostic procedures.*

**IBL IMMUNO BIOLOGICAL LABORATORIES**

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## 1. INTENDED USE

Enzymeimmunoassay for the in-vitro-diagnostic quantitative determination of cortisol in human serum or plasma.

## 2. INTRODUCTION

Cortisol /hydrocortisone, compound F) is the main corticosteroid secreted in humans by the adrenal cortex. This steroid hormone has a molecular weight of 363.5.

In most physiological conditions, only about 10% of plasma cortisol circulates unbound from transcortin and albumin. Among the products of the human adrenal cortex, only cortisol is involved in the regulation of ACTH secretion.

As the level of free (non-protein bound) cortisol in blood rises, the release of ACTH is inhibited by the negative feedback effect. Conversely, if cortisol levels are subnormal, the negative feedback decreases, ACTH levels rise, and the adrenal cortex secretes cortisol until normal blood levels are restored.

The release of ACTH is under control of hypothalamic corticotrophin-releasing hormone (CRH); the negative feedback system involving cortisol has been identified at both hypothalamic and pituitary levels. (1).

Normally during the day there is a fluctuation of cortisol achieving the highest level in the morning and the lowest in the night. Useful information is given when cortisol measurement is done in samples withdrawn at a fixed hour (8.00 a.m.).

The main biological effects of cortisol are: promotion of gluconeogenesis, deposition of liver glycogen, increase in blood glucose concentration when the carbohydrate utilization is reduced, effect on fat metabolism and anti-inflammatory action.

Cortisol measurement is a powerful tool for the evaluation of suspected abnormalities in glucocorticoid production: Cushing's Syndrome (hypercortisolism), Addison's disease or secondary adrenal insufficiency (hypocortisolism). In many cases, it is necessary to perform dynamic tests (suppression or stimulation) in order to localize the defect at one of the three main levels (i.e. adrenal, pituitary, hypothalamus).

## 3. PRINCIPLE OF THE TEST

The Cortisol 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 monoclonal antibody directed towards a unique antigenic site on the Cortisol molecule.

Endogenous Cortisol of a patient sample competes with a cortisol horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase conjugate is reverse proportional to the concentration of Cortisol in the sample. After addition of the the substrate solution, the intensity of colour developed is reverse proportional to the concentration of Cortisol in the patient sample.

## 4. PRECAUTIONS

1. This kit is for in vitro diagnostic use only.
2. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
3. 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.
4. Avoid contact with Stop Solution containing 0.5 M H<sub>2</sub>SO<sub>4</sub>. It may cause skin irritation and burns.
5. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
6. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
7. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
8. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
9. Do not use reagents beyond expiry date as shown on the kit labels.
10. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.
11. 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.
12. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.

13. Safety Data Sheets for this product are available upon request directly from IBL.
14. The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

## 5. KIT COMPONENTS

### 5.1. Contents of the Kit

1. **MTP** **Microtiterwells**, 12x8 (break apart) strips, 96 wells  
Wells coated with anti-Cortisol monoclonal antibody
2. **CAL** **Standard (Standard 0-6)**, 7 vials, 1 ml, ready to use  
Concentrations: 0, 20, 50, 100, 200, 400, 800 ng/ml,  
thus corresponding to 0, 55.2, 138, 276, 552, 1104, 2208 nmol/l, .  
*Conversion factor: 1 ng/ml = 2.76 nmol/l.*
3. **ENZCONJ** **Enzyme Conjugate**, 1 vial, 25 ml, ready to use  
Anti-Cortisol antiserum conjugated to horseradish peroxidase
4. **TMB SUBS** **Substrate Solution**, 1 vial, 14 ml, ready to use  
TMB
5. **TMB STOP** **Stop Solution**, 1 vial, 14 ml, ready to use  
contains 0.5M H<sub>2</sub>SO<sub>4</sub>  
Avoid contact with the stop solution. It may cause skin irritations and burns.
6. **WASHBUF** **Wash Solution**, 1 vial, 30 ml (40X concentrated)  
see „Preparation of Reagents“

**Note:** Additional *Standard 0* for sample dilution is available on request.

### 5.2. Equipment and material required but not provided

- A microtiterplate calibrated reader (450±10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Aqua dest.

### 5.3. Storage and stability of the Kit

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

All opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foilbag has been opened, care should be taken to close it tightly again.

### 5.4. Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

#### 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.*

### 5.5. Disposal of the Kit

The disposal of the kit must be made according to the national official regulations. Special information for this product are given in the Material Safety Data Sheets (see chapter 13).

### 5.6. Damaged Test Kits

In case of any severe damage of the test kit or components, IBL 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 stored until a final solution has been found. After this, they should be disposed according to the official regulations.

## 6. SPECIMEN

Serum or plasma (EDTA-, Heparin- or citrat plasma) can be used in this assay.  
Do not use haemolytic, icteric or lipaemic specimens.

### 6.1. Specimen Collection

#### Serum:

Collect blood by venipuncture (e.g Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature.

#### Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.

(E.g for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001; for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001; for Citrat plasma Sarstedt Monovette – green cap - # 02.167.001.)

### 6.2. Specimen Storage

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying.

Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

### 6.3. Specimen Dilution

If in an initial assay, a serum specimen is found to contain more than the highest standard, the specimens can be diluted 10-fold or 100 fold with *Standard 0* and reassayed 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 Serum + 90 µl Standard 0 (mix thoroughly)
- b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl Standard 0 (mix thoroughly).

## 7. TEST PROCEDURE

### 7.1. General Remarks

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipet tips for each standard, control or sample in order to avoid crosscontamination
4. 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.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature.

### 7.2. Assay Procedure

All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.

1. Secure the desired number of Microtiterwells in the holder.
2. Dispense **20 µl** of each Standard, controls and samples with new disposable tips into appropriate wells.
3. Dispense **200 µl** Enzyme Conjugate into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **60 minutes** at room temperature without covering the plate.
6. 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!

7. Add **100 µl** of Substrate Solution to each well.
8. Incubate for **15 minutes** at room temperature.
9. Stop the enzymatic reaction by adding **100 µl** of Stop Solution to each well.
10. Read the OD at **450±10 nm** with a microtiterplate reader **within 10 minutes** after adding the Stop Solution.

### 7.3. Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. 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: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
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. For the calculation of the concentrations this dilution factor has to be taken into account.

Below is listed a typical example of a standard curve with the Cortisol ELISA.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/ml)	2.30
Standard 1 (20 ng/ml)	1.67
Standard 2 (50 ng/ml)	1.24
Standard 3 (100 ng/ml)	0.87
Standard 4 (200 ng/ml)	0.57
Standard 5 (400 ng/ml)	0.35
Standard 6 (800 ng/ml)	0.23

## 8. QUALITY CONTROL

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 pathological 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 patient 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 IBL directly.

## 9. EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Cortisol values in serum or plasma ranges from 50 to 230 ng/ml (138-635 nmol/l) between 8:00 - 10:00 a.m., and from 30 to 150 ng/ml (82.8-414 nmol/l) at 4:00 p.m.

These values are from Tietz's Textbook (2) and may be used as main guideline.

## 10. ASSAY CHARACTERISTICS

### 10.1. Assay Dynamic Range

The range of the assay is between 0 – 800 ng/ml.

### 10.2. Specificity of Antibodies (Cross Reactivity)

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

Steroid	Crossreactivity
Cortisol	100%
Corticosterone	45%
Progesteron	< 9%
Deoxycortisol	< 2%
Dexamethazone	< 2%
Estriol	< 0.01%
Estrone	< 0.01%
Testosterone	< 0.01%

### 10.3. Analytical Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of *Standard 0* and was found to be 2.5 ng/ml (6.9 nmol/l).

### 10.4. Precision

#### Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (ng/ml)	CV (%)
1	20	43.5	8.1
2	20	226.5	3.2
3	20	403.6	5.6

#### Inter Assay Variation

The between assay variability is shown below:

Sample	Mean (ng/ml)	CV (%)
1	55	6.6
2	209	7.7
3	361	6.5

### 10.5. Recovery

Samples have been spiked by adding Cortisol solutions with known concentrations in a 1:1 ratio.

The expected values were calculated by addition of half of the values determined for the undiluted samples and half of the values of the known solutions. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Added Concentration 1:1 (v/v) (ng/ml)	Measured Conc. (ng/ml)	Expected Conc. (ng/ml)	Recovery (%)
1	--	57	57.0	100
	200	110	128.5	86
	400	216	228.5	95
	800	436	428.5	102
2	--	240	240	100
	200	210	220	95
	400	356	320	111
	800	514	520	99
3	--	378	378	100
	200	263	289	91
	400	355	389	91
	800	558	589	95

### 10.6. Linearity

Sample	Dilution	Mean Conc. (ng/ml)	Recovery (%)
1	None	48.0	--
	1:2	22.0	92
	1:4	12.9	108
	1:8	6.0	100
	1:16	3.3	110
2	None	255.0	--
	1:2	118.0	93
	1:4	63.1	99
	1:8	34.2	107
	1:16	15.9	100
3	None	427	--
	1:2	190	89
	1:4	97	91
	1:8	50	94
	1:16	25	94

## 11. LIMITATIONS OF USE

### 11.1. Interfering Substances

Any improper handling of samples or modification of this test might influence the results. Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 7.5 mg/ml) have no influence on the assay results.

### 11.2. Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Cortisol in a sample.

## 12. LEGAL ASPECTS

### 12.1. 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.

## 12.2. Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 10.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutical consequences.

## 12.3. 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.

## 13. REFERENCES

1. L. Thomas, Labor und Diagnose, 4. Auflage, 1992
2. Tietz, N.W., Textbook of Clinical Chemistry, Saunders, 1968

# Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	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:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
	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: / Fabricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>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.</p>	

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**LIABILITY:** Complaints will only be accepted in written and if all details of the test performance and results are included (complaint form available from IBL or supplier). 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. 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 kit during transportation is not subject to the liability of the manufacturer.