Instructions for Use

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IBL Products on the Internet

Cortisol ELISA

Cat.-No. : RE 520 61

Size : 12 x 8

Storage : 2 - 8 °C

Enzyme immunoassay (microtiter strips) for the quantitative determination of Cortisol in human serum and plasma

- For in-vitro diagnostic use only -

CE

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1. 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 the control of hypothalamic corticotrophin releasing hormone (CRH); the negative feedback system involving cortisol has been identified at both hypothalamic and pituitary levels.
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 Useful information is given when cortisol measurement is done in samples withdrawn at a fixed hour(8:00 a.m.).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 glucogenesis, deposition of liver glycogen, increase in blood glucose concentration when the carbohydrate utilisation 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 localise the defect at one of the three main levels (i.e. adrenal, pituitary, hypothalamus).

NOTE: Additional information about this assay (e.g. alternative applications) is available on the internet Internet (http://www.IBL-Hamburg.com) or upon request directly from IBL in Hamburg

2. Principle of the Test

The Cortisol ELISA kit provides materials for the quantitative measurement of Cortisol in human serum and plasma.
This assay is based on the competition principle and the microtiter plate separation. 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 an incubation the wells are washed to stop the competition reaction. Having added the TMB substrate solution the concentration of antigen is inversely proportional to the optical density measured. The measured Ods of the standards are used to construct a calibration curve against which the unknown samples are calculated.

3. Precautions

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- All reagents of this testkit 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.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All in the protocol indicated volumes (pipetting volumes and pretreatment steps) has to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.

- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.

- Safety Data Sheets for this product are available on the homepage of IBL or upon request directly from IBL-Hamburg. The Safety Data Sheets fit the demands of:
  - EU-Guideline 91/155 EWG
  - ISO-Standard 11014
  - ANSI-Standard
  - OSHA (US)

4. Kit Components

4.1 Contents of the Kit

Do components contain ≤ 250 µl solution, please care that all the solution is on the bottom of the vial.

4.1.1 Microtiter strips 12 x 8 wells
coated with anti cortisol antiserum
(rabbit, polyclonal) in foilbag with desiccant.

4.1.2 Reference Standard A (Zero Standard) 1 vial
2 ml, ready to use of stripped human serum containing 0.1% thimerosal and 0.3% proclin.

4.1.3 Reference Standard Set (B - G) 6 vials
0.5 ml each, ready to use containing the below mentioned concentrations of testosterone in protein matrix and 0.1% thimerosal, 0.3% proclin.

Standard	**B**	**C**	**D**	**E**	**F**	**G**
Concentration in ng/ml	20	50	100	200	400	800
Concentration in nmol/l	55,2	138	276	552	1104	2280

The concentration of cortisol in the standards has been validated by HPLC after solvent extraction.

4.1.4 Enzyme Conjugate 1 vial
22 ml, ready to use Cortisol conjugated to the enzyme horseradish peroxidase supplemented by enzyme stabilisers and 0.3% proclin

4.1.5 TMB Substrate Solution (-LS-) 1 vial
22 ml, ready to use containing a solution of tetramethylbenzidine (TMB) with hydrogen peroxide in buffer supplemented by stabilisers.

4.1.6 TMB Stop Solution 1 vial
11 ml, ready to use 0.5 M Hydrochloric acid (HCl). Avoid contact with stop solution it may cause skin irritations and burns.

4.1.7 Wash Buffer, concentrate (40x) 1 vial

30 ml, concentrate containing phosphate buffered saline with a nonionic detergent dilute 1 to 40 with distilled water prior to use

Material required but not provided:

- Automatic pipettes to dispense 5, 50, 100 and 500 µl (a multichannel pipetting device such as Titertek is suitable for adding reagents to the wells.)
- Distilled water.
- Microtiterplate photometer (ELISA reader) with 450 nm filter and 620 nm reference wave lenght.
- Commercial controls for cortisol. They should contain high and low levels and should be included as unknowns in every assay performed with this kit. Control values obtained in each run must fall within previously established ranges.

4.2 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.
Once the foilbag of the microtiter strips has been broken care should be taken to close it tightly again. The immuno reactivity of the coated microtiter wells is stable for approx. 8 weeks in the broken, but tightly closed plastic zip pouch containing the desiccant.

4.3 Preparations of Reagents

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

4.3.1 Wash Buffer

10 ml of the concentrate have to be diluted 1:40 with bidistilled water up to 400 ml. This gives the ready for use Wash Buffer.

4.4 Storage and Stability of prepared Reagents

4.4.1 Wash Buffer

After opening the wash buffer is stable at 2-8°C for up to 8 weeks or up to the expiry date respectively.

4.4.2 Reference Standard Set

After opening the standards are stable at 2-8°C for up to 8 weeks or up to the expiry date respectively.

4.4.3 Enzyme Conjugate

After opening the enzyme conjugate is stable at 2-8°C for up to 8 weeks or up to the expiry date respectively.
Black particles may occur in the vial. This has no influence on the test result.

4.4.4 TMB Substrate Solution

After opening the TMB substrate solution is stable at 2-8°C for up to 8 weeks or up to the expiry date respectively.
The substrate solution is colorless or weak yellowish-green colored. After light exposition there may occur a blue color, which should disappear when stored in the darkness. If the blue color does not disappear, the substrate solution is unserviceable.

4.5 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 3).

4.6 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 use 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. Specimen

5.1 Collection

The usual precautions for venipuncture should be observed. Do not use grossly hemolytic, icteric or grossly lipemic specimens.

5.2 Storage

Serum or EDTA plasma should be used, and the usual precautions for venipuncture should be observed. The specimen may be stored at 2 - 8 °C for up to 24 hours. For a longer storage, the samples should be frozen at -20 °C or lower.

5.3 Pretreatment

No special sample pretreatment is necessary. Samples suspected to contain cortisol concentration higher than 800 ng/ml have to be diluted with zero standard. The result obtained has to be corrected by the dilution factor.

6. Test Procedure

GENERAL REMARKS:

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 disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination.

Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

6.1 Assay Procedure

6.1.1 Secure the desired number of coated microtiter strips in the holder.

6.1.2 Pipet **20 µl of standards, controls and unknowns** into the appropriate wells of the strips.

6.1.3 Add **200 µl of enzyme conjugate** into each well. Thoroughly mix the plate for 10 seconds. It is important to have complete mixing in this step.

6.1.4 **Incubate for 60 minutes** at room temperature (18 - 24 °C) without covering the plate.

6.1.5 **Washing**: discard the incubation solution, rinse the wells 3x with 300 µl wash buffer and remove any residual. Strike the wells sharply on absorbent paper to remove residual droplets.

6.1.6 Promptly pipet **100 µl of the TMB substrate solution** into the rinsed wells at timed intervals.

6.1.7 **Incubate for 15 minutes** at room temperature (18 - 24 °C).

6.1.8 Stop the reaction by adding **100 µl** of **TMB stop solution** to each well at timed intervals.

6.1.9 **Shake gently** the microtiterplate being careful not to let the content come from the wells and **read at 450 ± 10 nm** within 60 minutes from the stopping.

6.2. Calculation of Results

On a semilogarithmic graph paper the concentration of the standards (abscissa, logarithmic) are plotted against their corresponding optical density (ordinate, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/ODmax, and then plotted on the ordinate.

Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.

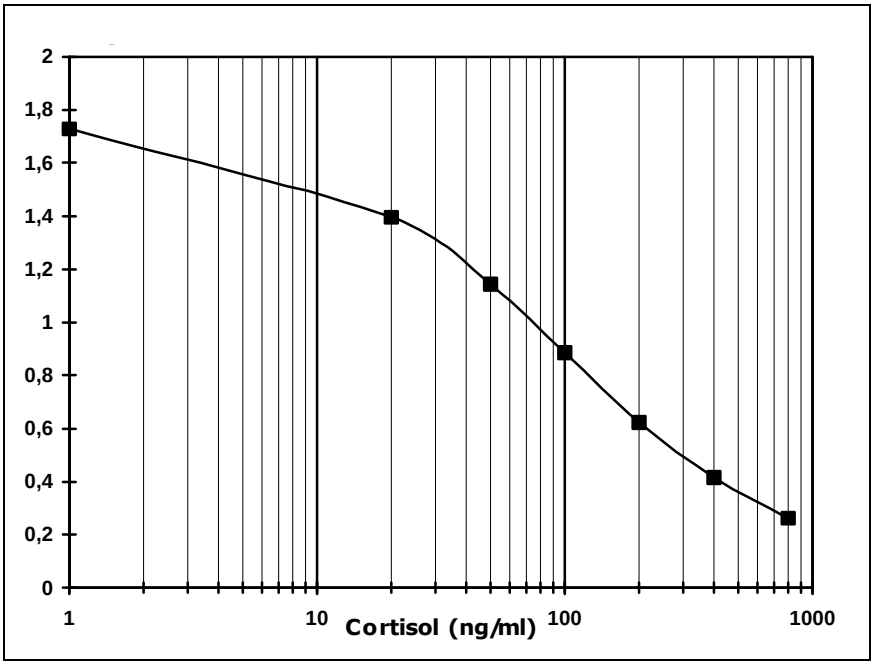
The concentration of the samples can be read directly from this standard curve by using their average optical density.

For the accuracy of the results obtained see chapter 7 (Assay Characteristics).

Any sample reading greater than the highest standard should be diluted appropriately with zero standard and reassayed. The result has to be multiplied by the corresponding dilution factor.
Below is listed a typical example of a standard curve with the Cortisol ELISA.

Standard concentration	O.D.	OD/ODmax.
0 ng/ml	1.728	----
20 ng/ml	1.396	80.6
50 ng/ml	1.142	65.8
100 ng/ml	0.886	50.8
200 ng/ml	0.624	35.6
400 ng/ml	0.416	23.4
800 ng/ml	0.263	14.4

Typical Calibration Curve
Cortisol ELISA



6.3 Automation

Experiments in the IBL laboratories have shown that the Cortisol ELISA is suitable to run on an automated ELISA processor. The protocol is available upon request at IBL directly or can be downloaded from our internet website chapter Special Information About the Product “Automation”.

7. Assay characteristics

7.1 Expected values

The following values can be used as preliminary guidelines until each laboratory establishes its own normal ranges. Cortisol value 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/Ll at 4:00 p.m. These values are from Tietz's Textbook and may be used as main guidelines until each laboratory establishes its own normal ranges.

	ng/ml
8.00 - 10.00 A.M.	50 - 230
4.00 P.M.	30 - 150

Conversion factor: 1 ng/ml = 2.76 nmol/l

7.2 Specificity

The specificity of the testosterone assay was assessed according to Abraham's method. The percentage indicate cross-reactivity at 50 % displacement compared to cortisol.

Steroid	**Cross-reactivity**
Cortisol	100.0 %
Prednisolone	60.0 %
Corticosterone	29.0 %
Cortisone	3.0 %
17-OH Progesterone	< 0.5 %
11-Deoxycortisol	< 1.0 %
Prednisone	< 0.1 %
Progesterone	< 0.1 %
Dexamethazone	< 0.1 %

Steroid	Cross-reactivity
Desoxycorticosterone	< 0.1 %
Dehydroepiandrosterone sulphate	< 0.1 %
Estriol	< 0.1 %
Estrone	< 0.1 %
Testosterone	< 0.1 %
Androstenedione	< 0.1 %

7.3 Sensitivity

The lower limit of detection - defined as the cortisol concentration given by the mean absorbance of the zero calibrator minus 2 standard deviations - was assessed to be approx. 2.5 ng/ml (6.9 nmol/l).

7.4 Precision

7.4.1. Intra Assay Variation

Within run variation was determined by replicate determination of 3 different control sera in one assay. The within assay variability is shown below:

Sample	n	Mean (ng/ml)	Standard deviation (ng/ml)	c.v. (%)
1	18	26.68	1.18	4.41
2	20	172.86	6.87	3.98
3	20	278.80	13.11	4.70

7.4.2. Inter Assay Variation

Between run variation was determined by replicate measurement of 3 different control sera in several different assays. The between assay variability is shown below:

Sample	n	Mean (ng/ml)	Standard deviation (ng/ml)	c.v. (%)
1	24	26.14	1.30	4.96
2	24	167.96	9.59	9.59
3	26	266.92	17.68	6.62

7.5 Accuracy

7.5.1. Linearity

3 samples having different Cortisol levels were serially diluted with Zero Standard and the Cortisol contents were assayed in the diluted samples by the ELISA. 3 dilutions were performed for each sample.

Sample	Dilution	Measured conc. (ng/ml)	Expected conc. (ng/ml)	Recovery (%)
1	--	287.27		
	1:2	136.87	139.14	98.37
	1:4	69.21	69.57	99.49
	1:8	31.84	34.78	91.54
2	--	259.61		
	1:2	128.99	129.81	99.37
	1:4	65.77	64.90	101.34
	1:8	30.98	32.45	95.47
3	--	157.94		
	1:2	83.00	78.97	105.10
	1:4	40.89	39.49	103.56
	1:8	23.20	19.74	117.51

7.5.2. Recovery

Normal human serum with known concentration was enriched with increasing amounts of Cortisol (all results given in ng/ml).

Sample	Added conc.	Expected conc.	Measured conc.	% Recovery
1 (endogene conc.: 84 ng/ml)	0.0		84.00	100
	50	134.00	133.66	99.75
	100	184.00	170.15	92.47
	200	284.00	270.48	95.24
2 (endogene conc.: 14.90 ng/ml)	0.0		14.90	100
	50	64.90	66.73	102.82
	100	114.90	109.72	95.49
	200	214.90	202.91	94.42

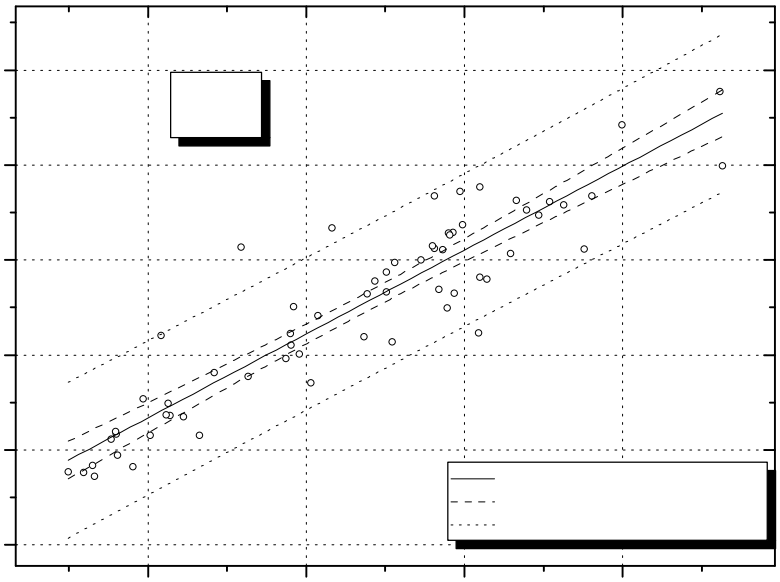
7.5.3 Method comparison

Comparison Cortisol ELISA vs. RIA

63 patient samples were assayed by the IBL ELISA procedure and a commercially available RIA kit.

Linear regression gives the following statistics:

IBL ELISA = 0.88 x RIA + 22.71; r = 0.92 ; n = 63



7.5.4 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 kit controls and the corresponding results of the IBL QC-Laboratory are stated in the QC certificate added to the kit. The values 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-Hamburg directly.

8. Limitations of Use

8.1 Interfering Substances

Any improper handling of samples or modification of this test might influence the results. Interferences caused by improper sample handling are explained in the chapters ‘Specimen - Collection’.

Azide and thimerosal at concentrations higher than 0.1 % interfere in this assay. Therefore control sera or samples containing higher concentrations of the above mentioned components may give false results.

Some medications influence the metabolism or some diets contain serotonin and other biogenic amines in relatively high quantities. They may interfere with the test result. For further details see chapter ‘’Assay Characteristics, Expected values’’.

8.2 High-Dose-Hook Effect

There exists no High-Dose-Hook effect in a competitive assay. In case of sandwich assays the probability of a High-Dose-Hook effect is reduced if the antibody and antigen containing solutions are added in a sequential order.

9. Legal Aspects

9.1 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-Hamburg.

9.2 Complaints

Complaints will only be accepted in written format (preferably on the manufacturer's complaint form) and only if all details of the test kit, as well as the test results, are included. A copy of the complaint form is available from IBL-Hamburg upon request.

9.3 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 9.1. 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.

9.4 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 9.3. 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.

10. References

1. Tietz, NW: Textbook of Clinical Chemistry. Saunders 1986.
2. L Thomas, Labor und Diagnose, 4 Auflage, 1992

IBL HAMBURG CE	**Cortisol ELISA** Short Instructions for Use	**Cat. No.** **RE 52061** Size: 12 x 8 Storage: 2 - 8 °C

1. Preparation of Reagents

- **Wash Buffer:** Dilute the concentrate 1:40 with bidist. water (e.g. 10 ml of concentrate + 390 ml of bidist. water). Store at 2-8°C for 8 weeks.

2. Specimen Collection and Storage

Sample: Serum, Plasma,
Storage: up to 24 h at 2-8°C, longer storage at ≤ –20°C.

3. Assay Procedure

Storage of reagents at 2 - 8 °C. Allow reagents to reach room temperature.

⇩	Pipet **20 µl of Standards, controls** and **samples**.
⇩	Add **200 µl of Enzyme Conjugate** to each well. Mix thoroughly for **10 sec**.
⌛	Incubate **60 minutes at room temperature (18 – 24 °C)** without covering the plate.
≈	Wash* **3 x with Wash Buffer**.
⇩	Pipet** **100 µl of TMB-Substrate Solution.**
⌛	Incubate **15 min. at room temperature**.
⇩	Pipet** **100 µl of TMB-Stop Solution.**
🖳	Briefly mix contents, read the optical density at **450 nm** (reference wave lenght 600-650 nm) within **60 min.** after stopping.

* Wash procedure is essential for the assay results.
** Stop solution should be pipetted after incubation in the same time intervals as the substrate solution.

The concentrations of the standards (abscissa, logarithmic) are plotted against their corresponding optical density (ordinate, linear) on a semilogarithmic graph paper (standard curve). The concentration of the samples and controls can be read directly from this standard curve.

4. Expected values

It is recommended that each laboratory establishes its own range of normal values. The following values may serve as a guide line:

8.00a.m. – 10.00a.m. : 50 – 230 ng/ml (138 – 635 nmol/l)
4.00p.m.: 30 – 150 ng/ml (83 – 414 nmol/l)
Conversion factor: 1 ng/ml = **2,76** nmol/l

Attention: For comprehensive information about the test procedure please refer to the detailed instructions for use available on the Internet (http://www.IBL-Hamburg.com) or upon request from IBL in Hamburg.

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