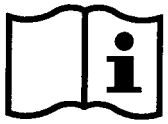
Product information

Information about other products is available at: www.ibl-america.com



Cortisol ELISA

RUO

REF **IB79343**

Σ **96**

For Research Use Only – Not for Use in Diagnostic Procedure

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955 Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com Web: www.ibl-america.com

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1 INTRODUCTION

1.1 Intended Use

The **IBL-America Cortisol ELISA** is a competitive immunoassay for the quantitative measurement of cortisol in serum and plasma (EDTA, Li-Heparin).
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1.2 Description of the analyte

Cortisol is a corticosteroid hormone or glucocorticoid produced by the adrenal cortex that is part of the adrenal gland (in the Zona fasciculata and the Zona reticularis of the adrenal cortex). It is usually referred to as the "stress hormone" as it is involved in response to stress.
90% of the cortisol is bound to cortisol-binding globulins (CBG), around 7% to Albumin and the rest is free (1). 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 corticotropin-releasing hormone (CRH); the negative feedback system involving cortisol has been identified at both hypothalamic and pituitary levels.

2 PRINCIPLE

The IBL-America 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 an anti-cortisol antibody. An unknown amount of cortisol present in the 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 inversely proportional to the concentration of cortisol in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of cortisol in the sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in research use only – Not for use in diagnostic procedures.
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. All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the reagents must be handled in the same manner as potentially infectious material.
4. The microplate contains break-apart strips. Unused wells must be stored at 2-8°C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples 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 substrate solution that had previously been used for 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 washing steps.
9. Allow the reagents to reach room temperature (18-25°C) before starting the test. Temperature will affect the absorbance readings of the assay.
10. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
12. Wear disposable protective gloves when handling samples and reagents. Microbial contamination of reagents or samples 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 be slightly different.
17. Avoid contact with Stop Solution. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, CMIT and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
20. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from IBL-America.
21. If product information, including labeling, is incorrect or inaccurate, please contact the kit manufacturer or supplier.

4 REAGENTS

4.1 Reagents provided

1. **SORB MT Microtiter Plate**, 12 x 8 (break-apart) strips with 96 wells; wells coated with anti-cortisol antibody.
2. **CAL 0 – 5 Calibrators (Calibrator 0-5),** 6 vials, 0.3 ml each, ready to use;
   contain cortisol in human serum. Concentrations: 0 - 10 - 30 - 90 - 270 - 800 ng/ml.
3. **CONTROL 1 & 2 Control 1 (low) / Control 2 (high)**, 2 vials, 0.3 ml each, ready to use; contain cortisol in human serum. For control values and ranges please refer to QC-Datasheet.
4. **ENZ CONJ Enzyme Conjugate**, 1 vial, 22 ml, color: red, ready to use; horseradish peroxidase-labeled cortisol in buffered matrix.
5. **SUB TMB Substrate Solution**, 1 vial, 22 ml, ready to use; contains Tetramethylbenzidine (TMB).
6. **STOP SOLN Stop Solution**, 1 vial, 7 ml, ready to use; contains 2 N hydrochloric acid solution. Avoid contact with the Stop Solution. It may cause skin irritations and burns.
7. **WASH SOLN 10x Wash Solution**, 1 vial, 50 ml (10x concentrated); see "**Reagent Preparation" (4.4).**

4.2 Materials required but not provided

- A microtiter plate reader capable for endpoint measurement at 450 nm
- Calibrated variable precision micropipettes and multichannel pipettes with disposable pipette tips
- Microplate mixer operating at 900 rpm
- Manual or automatic equipment for microtiter plate washing
- Absorbent paper
- Distilled or deionized water
- Timer
- Semilogarithmic graph paper or software for data reduction

4.3 Storage conditions

When stored at 2-8°C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8°C. After first opening the reagents are stable for 30 days if used and stored properly. Keep away from heat and direct sunlight.
Microtiter wells must be stored at 2-8°C. Take care that the foil bag is sealed tightly.

4.4 Reagent preparation

Allow the reagents and the required number of wells to reach room temperature (18-25°C) before starting the test.

Wash Solution:
Dilute 50 ml of 10x concentrated Wash Solution with 450 ml deionized water to a final volume of 500 ml. The diluted Wash Solution is stable for at least 12 weeks at room temperature (18-25°C). Precipitates may form when stored at 2-8°C, which should dissolve again by swirling at room temperature (18-25°C). The Wash Solution should only be used when the precipitates have completely dissolved.

4.5 Disposal of the kits

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged test kits

In case of any severe damage of the test kit or components, IBL-America has to be informed in writing within 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 SAMPLE COLLECTION AND STORAGE

For determination of cortisol **serum and plasma (EDTA, Li-Heparin)** can be used.
The usual precautions for venipuncture should be observed (1). It is important to preserve the chemical integrity of a blood sample from the moment it is collected until it is assayed. Do not use hemolytic, icteric or lipemic samples. Furthermore, we recommend special caution when using gel collection systems, as an influence on the measurement results cannot be excluded in case of improper handling. Samples containing sodium azide should not be used in the assay.
The procedure calls for 10 µl sample per well. The samples should be assayed immediately or aliquoted and stored at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples expected to contain cortisol concentrations higher than the highest calibrator (800 ng/ml) must be diluted with the zero calibrator before assay. The additional dilution step has to be taken into account for the calculation of the results.

6 ASSAY PROCEDURE

6.1 General remarks

- All reagents and samples 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 standard, control or sample in order to avoid cross-contamination.
- Optical density 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.
- Respect the incubation times as stated in this instructions for use.
- Calibrators, controls, and samples should at least be assayed in double determinations.
- Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or a multistepper, respectively, 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 solution, and that there are no residues in the wells.
- A calibrator curve must be established for every run.

6.2 Assay procedure

1. Prepare a sufficient number of microplate wells to accommodate Calibrators and Samples in duplicates.
2. Dispense **10 µl** of each **Calibrator, Sample and Control** with new disposable tips into appropriate wells.
3. Dispense **200 µl** of **Enzyme Conjugate** into each well.
4. Incubate for **60 minutes** at room temperature (18-25°C) on a plate shaker (900 rpm).
5. Discard the content of the wells and rinse the wells **4 times** with diluted **Wash Solution** (300 µl per well). Remove as much Wash Solution as possible by beating the microplate on absorbent paper.
   Important note:
   The sensitivity and precision of the assay is markedly influenced by the correct performance of the washing procedure!
6. Add **200 µl** of **Substrate Solution** to each well.
7. Incubate without shaking for **30 minutes** at room temperature (18-25°C) in the dark.
8. Stop the reaction by adding **50 µl** of **Stop Solution** to each well.
9. Determine the optical density of each well at 450 nm and read the wells within 15 minutes.

6.3 Calculation of results

1. Calculate the average absorbance values for each set of calibrators, controls and samples.
2. The obtained optical density of the standards (y-axis, linear) are plotted against their corresponding concentrations (x-axis, logarithmic) either on semilogarithmic paper or using an automated method.
3. Using the mean optical density value for each sample, determine the corresponding concentration from the calibration curve.

4. Automated method: The results in the package insert have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

7 QUALITY CONTROL

Good laboratory practice requires that controls are 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.
The use of control samples is advised to assure the day-to-day validity of results. The controls and the corresponding results of the QC laboratory are stated in the QC certificate included in the kit. The values and ranges stated on the QC certificate always refer to the current kit lot and should be used for direct comparison 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 results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices, microtiter plate reader, 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 Analytical Sensitivity

The lowest analytical detectable level of cortisol that can be distinguished from the Zero Calibrator is 0.38 ng/ml at the 2SD confidence limit.

8.2 Specificity (Cross Reactivity)

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to cortisol.

Steroid	% Cross reaction
Pregnenolone	<0.3%
Estrone	<0.3%
Estradiol	<0.3%
DHEA	<0.3%
17-Hydroxyprogesterone	0.8%
Prednisolone	100%
Testosterone	<0.3%
Cortisone	76.9%
Corticosterone	0.4%
Danazole	<0.3%
Androstenedione	<0.3%
Prednisone	70%
11-Deoxycortisol	39.9%
Estriol	<0.3%
Dexamethasone	<0.3%
11-Deoxycorticosterone	<0.3%
Progesterone	<0.3%
DHEA-S	<0.3%

8.3 Assay dynamic range

The range of the assay is between 10 - 800 ng/ml.

8.4 Reproducibility

8.4.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of three serum samples within one run. The within-assay variability is shown below:

	Serum 1	Serum 2	Serum 3
Mean (ng/ml)	60.9	102.1	264.1
SD	4.2	6.9	19.0
CV (%)	7.0	6.8	7.2
n =	20	20	20

8.4.2 Inter-Assay

The inter-assay (between-run) variation was determined by duplicate measurements of three serum samples in 10 different tests.

	Serum 1	Serum 2	Serum 3
Mean (ng/ml)	57.3	106.7	287.3
SD	3.0	8.3	26.3
CV (%)	5.2	7.8	9.2
n =	10	10	10

8.5 Recovery

Recovery was determined by adding increasing amounts of the analyte to three different samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

Sample	Spiking (ng/ml)	Measured (ng/ml)	Expected (ng/ml)	Recovery (%)
1	native	204.3	-	-
	100	306.2	304.3	101%
	200	394.1	404.3	97%
	300	527.7	504.3	105%
2	native	76.4	-	-
	100	186.7	176.4	106%
	200	272.8	276.4	99%
	300	415.5	376.4	110%
3	native	110.3	-	-
	100	222.6	210.3	106%
	200	329.3	310.3	106%
	300	440.5	410.3	107%

8.6 Linearity

Four serum samples containing different amounts of analyte were serially diluted with Calibrator 0 and assayed. The percentage linearity was calculated by comparing the expected and measured values.

Serum	Dilution	Measured (ng/ml)	Expected (ng/ml)	Linearity (%)
1	native	465.8	-	-
	1 : 2	232.0	232.9	100%
	1 : 4	100.5	116.4	86%
	1 : 8	64.1	58.2	110%
2	-	224.4	-	-
	1 : 2	132.9	112.2	118%
	1 : 4	70.7	56.1	126%
	1 : 8	36.2	28.1	129%
3	native	226.3	-	-
	1 : 2	136.6	113.1	121%
	1 : 4	69.9	56.6	123%
	1 : 8	36.0	28.3	127%
4	native	321.7	-	-
	1 : 2	179.7	160.8	112%
	1 : 4	92.1	80.4	115%
	1 : 8	41.9	40.2	104%

9 LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with complete understanding of the package insert instruction and adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

9.1 Interfering Substances

- Hemoglobin (up to 1000 mg/dl), Bilirubin (up to 40 mg/dl), and Lipids (up to 30 mg/ml) show no significant influence on the assay results. However, we recommend not to use any hemolytic, icteric or lipemic samples to avoid any interferences.
- Samples containing sodium azide should not be used in the assay.
- The result of any immunological test system may be affected by heterophilic antibodies, anti-species antibodies or rheumatoid factors present in human samples (2-4). For example, the presence of heterophilic antibodies in subjects who are regularly exposed to animals or animal products may interfere with immunological tests. Therefore, interference with this in vitro immunoassay cannot be excluded. If unplausible results are suspected, they should be considered invalid and verified by further testing.

9.2 Drug Interferences

Any medication (cream, oil, pill, etc.) containing cortisol will significantly influence the measurement of this analyte. The same is true for any medication containing Prednisolone and Cortisone. Any medication should be taken into account when assessing the results.

9.3 High Dose Hook Effect

"High Dose Hook Effect" is not detected in the range between 0 – 10,000 ng/ml.

10 LEGAL ASPECTS

10.1 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 a sufficient number of controls within the test procedure 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.

10.2 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.
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 REFERENCES

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2. Marks V.: False-Positive Immunoassay Results: A Multicenter Survey of Erroneous Immunoassay Results from Assays of 74 Analytes in 10 Donors from 66 Laboratories in Seven Countries *Clinical Chemistry* 2002, 48:11: 2008-2016
3. Tate & Ward (2004) Interferences in Immunoassays, *Clin. Biochem Rev* Vol 25, May 2004
4. Selby (1999): Interference in immunoassays; *Ann. Clin. Biochem* 1999, 36: 704-721

SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Française	Español	Italiano
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tenga en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta