Instructions for Use

Cortisol Urine ELISA

Enzyme immunoassays for the quantitative determination of cortisol in urine.

REF **RE52241**

Σ **96**

2-8°C

EU: IVD CE

I B L I N T E R N A T I O N A L G M B H

Flughafenstrasse 52a
D-22335 Hamburg, Germany

Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11

IBL@IBL-International.com
www.IBL-International.com

1 INTENDED USE

The Cortisol Urine ELISA is a competitive immunoenzymatic colorimetric method for quantitative determination of free cortisol concentration in Urine.

2 CLINICAL SIGNIFICANCE

Cortisol is a steroid hormone released from the adrenal cortex in response to a hormone called ACTH (produced by the pituitary gland), it is involved in the response to stress; it increases blood pressure, blood sugar levels, may cause infertility in women, and suppresses the immune system.

Cortisol acts through specific intracellular receptors and has effects in numerous physiologic systems, including immune function, glucose-counter regulation, vascular tone, substrate utilization and bone metabolism. Cortisol is excreted primarily in urine in an unbound (free) form.

Cortisol is bound, in plasma, from corticosteroid-binding globulin (CBG, transcotin), with high affinity, and from albumin. Only free cortisol is available to most receptors.

These normal endogenous functions are the basis for the physiological consequences of chronic stress - prolonged cortisol secretion causes muscle wastage, hyperglycaemia, and suppresses immune / inflammatory responses. The same consequences arise from long-term use of glucocorticoid drugs.

The free cortisol fraction represents the metabolically active cortisol. In normal conditions, less then 1% it comes excrete in urines. In pathological conditions (syndrome of Cushing) the levels of free urinary cortisol are elevate, because the CBG don't bound the plasmatic cortisol in excess and it was remove with urines.

During pregnancy or estro-progestogen treatment an increase of plasmatic cortisol caused by an increment of the production of the transport protein, but the levels of free urinary cortisol results normal to indicate correct adrenal functionality.

This test is very useful to estimate the real adrenal function, because it measure the free cortisol, it is the metabolically active form. Moreover the measurement of free urinary cortisol is the better parameter for the diagnosis of the Cushing's syndrome.

3 PRINCIPLE

The Cortisol (antigen) in the sample competes with the antigenic Cortisol conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti Cortisol coated on the microplate (solid phase).
After incubation, the bound/free separation is performed by a simple solid-phase washing. Then, the enzyme HRP in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blu color that changes into yellow when the Stop Solution (H_2SO_4) is added.
The colour intensity is inversely proportional to the Cortisol concentration of in the sample.
Cortisol concentration in the sample is calculated through a calibration curve.

4 REAGENTS, MATERIAL AND INSTRUMENTATION

4.1 Reagents and material supplied in the kit

1.	**Coated Microplate** Anti-Cortisol IgG adsorbed on microplate	**MTP**	(1 microplate breakable)
2.	**Conjugate** Cortisol-HRP conjugate	**CONJ**	1 x 33 mL
3.	**Cortisol Standard 0**	**CAL 0**	1 x 4mL
4.	**Cortisol Standards 1 – 4**	**CAL1-4**	1 x 4 x 1mL
5.	**Washbuffer** (10x) Phosphate buffer, ProClin < 0.0015%	**WASHBUF** **CONC**	1 x 50 mL
6.	**TMB Substrate** H_2O_2-TMB 0.26g/L (avoid any skin contact)	**TMB SUBS**	1 x 15 mL
7.	**Stop Solution** Sulphuric acid 0.15 mol/L (corrosive: avoid any skin contact)	**TMB STOP**	1 x 15 mL
8.	**Control Low**	**CONTROL LOW**	1 x 1 mL
9.	**Control High**	**CONTROL HIGH**	1 x 1 mL

4.2 Reagents necessary which are not supplied

Distilled water.

4.3 Auxiliary materials and instrumentation

Automatic dispenser.
Microplate reader (450nm, 620-630 nm)

Notes

Store all reagents at 2 °C to 8 °C in the dark.
Open the bag of Microplate only when it is at room temperature and close immediately after use; once opened, the microplate is stable until the expiry date of kit.

5 WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents contain small amounts of ProClin 300 as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This method allows the determination of Cortisol from 10 ng/mL to 500 ng/mL.
- The clinical significance of the Cortisol determination can be invalidated if the patient was treated with corticosteroids or natural or syntetic steroids.

6 PRECAUTION

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying control samples.
- Maximum precision is required for reconstitution and dispensation of reagents.
- Plate readers measure vertically. Do not touch the bottom of the wells.

7 PROCEDURE

7.1 Preparation of the Calibrators (CAL 0 – CAL 4)

Before use, leave 5 minutes on a rotating mixer.

The Calibrators are ready to use and have the following concentration of Cortisol:

	CAL 0	CAL 1	CAL 2	CAL 3	CAL 4
ng/mL	0	10	50	150	500

Once opened, the Calibrators are stable 6 months at 2-8°C.

7.2 Preparation of the Conjugate

The Conjugate is ready to use.

Once opened, it stable 6 months at 2-8°C.

7.3 Preparation of the Sample

The determination of Cortisol with this kit should be performed in urine samples.

Important note: the kit has been designed to be used on untreated urine samples; acidification treatments of the urine that lead the pH to values below 5.0 could interfere with the assay and produce aberrant results.

It is not necessary to dilute the sample.

The total volume of urine excreted during a 24 hours should be collected and mixed in a single container.

Urine samples which are not to be assayed immediately should be stored at 2-8°C (≤48h) or at -20°C (≤ 6 months).

The Controls are ready to use.

In case of samples with concentration greater than 500 ng/mL dilute with Calibrator 0 (consider this dilution in the calculation of final concentration).

7.4 Preparation of Wash Solution

Dilute the content of each vial of the "10X Conc. Wash Solution" with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

In concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

7.5 Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (CAL 0 - CAL 4), two for each Control, two for each sample, one for Blank.

	Std. / Ctr.	Sample	Blank
Standards CAL 0 –CAL 4 Control	10 µL	-	-
Sample	-	10 µL	-
Conjugate	300 µL	300 µL	-
Mix well.Incubate at 37°C for 1 hour.			
Remove the contents from each well. Wash the wells 3 times with 350 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: in case you use an automatic washer, it is advised to do 6 washing steps.			
TMB-Substrate	100 µL	100 µL	100 µL
Incubate at room temperature (22°C to-28°C) for 15 minutes in the dark.			
Stop Solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 min.			

8 QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Urinary Cortisol for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

9 RESULTS

9.1 Mean Absorbance

Calculate the mean of the absorbance for each point of the calibration curve and of each sample.

9.2 Calibration curve

Plot the values of absorbance of the Calibrators against concentration. Draw the best-fit curve through the plotted points (es: Four Parameter Logistic).

9.3 Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in ng/mL.

To calculate the cortisol concentration in urine, calculate as above and correct for total volume of volume of urine collected in 24 hours: **ng/mL x Vol(mL) urine 24 h/ 1000 = µg Cortisol/24h**

10 REFERENCE VALUES

The urinary Cortisol concentration during the 24 hours are included in the following range: **50 - 190 µg / 24 hours**

11 PERFORMANCE CHARACTERISTICS

11.1 Precision

11.1.1 Intra Assay Variation

Within run variation was determined by replicate (20x) the measurement of three different urine samples in one assay. The within assay variability is ≤ 6.5%.

11.1.2 Inter Assay Variation

Between run variation was determined by replicate (10x) the measurement of three different urine samples in different lots of kit. The between assay variability is ≤ 7.2%.

11.2 Accuracy

The recovery of 12.5 - 25 - 50 - 100 ng/mL of Urinary Cortisol added to a sample gave an average value (±SD) of 107.48% ± 8.16% with reference to the original concentrations.

11.3 Sensitivity

The lowest detectable concentration of Urinary Cortisol that can be distinguished from the zero standard is 2.95 ng/mL at the 95 % confidence limit.

11.4 Specifity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Cortisol	100 %
Prednisolone	46.2 %
11-Deoxycortisol	4 %
Cortisone	3.69 %
Prednisone	3.10 %
11αOH Progesterone	1 %
Progesterone	< 0.1 %
Aldosterone	< 0.1 %
Pregnenolone	< 0.1 %
17b Estradiol	< 0.1 %
Estrone 3-solfato	< 0.1 %
Estriol	< 0.1 %
Testosterone	< 0.1 %
Spironolactone	< 0.1 %
DHEA	< 0.1 %
DHEA-S	< 0.1 %
Androstenedione	< 0.1 %
Androsterone	< 0.1 %
DHT	< 0.1 %
Danazol	< 0.1 %
Cholesterol	< 0.1 %
Dexamethasone	< 0.1 %

11.5 Correlation

The new Urinary Cortisol ELISA kit was compared to the old Urinary Cortisol ELISA kit. 100 urine samples were analysed.

The linear regression curve was calculated:

$y = 0.90^* x + 9.95$

$r^2 = 0.836$

12 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

13 BIBLIOGRAPHY

1. Foster, L.B. and Dunn, R.T. Clin Chem: 20/3, 365 (1974)
2. De Laceda, L., Kowarski, A., and Migeon, C.J. J. Clin Endocr. and Metab: 36:227 (1973)
3. Rolleri, E., Zannino, M., Orlandini, S., Malvano, R. Clin chim Acta 66 319 (1976)
4. Kobayashi, Y., et al. Steroids, 32 no. 1 (1978)
5. Arakawa, H., Maeda, M., Tsuji, A. Anal. Biochem. 97248 (1979)

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.


IBL International GmbH
Flughafenstr. 52A, 22335 Hamburg, Germany

Tel.: + 49 (0) 40 532891 -0 Fax: -11
E-MAIL: IBL@IBL-International.com
WEB: http://www.IBL-International.com