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DCM018-14
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URINARY CORTISOL ELISA

for routine analysis

Direct immunoenzymatic determination of free Cortisol in urine

IVD | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO018

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1. INTENDED PURPOSE

For *In Vitro* Diagnostic Use
For Laboratory Professional Use

Urinary Cortisol ELISA is a manual *in vitro* diagnostic device intended for the quantitative determination of free Cortisol in human urine from an adult population.

2. CLINICAL SIGNIFICANCE

Cortisol is a steroid hormone released from the adrenal cortex in response to an 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 than 1% is excreted in urine. In pathological conditions (Cushing syndrome), free urinary cortisol levels are very high as excess plasma cortisol doesn't not bind to the CBG, so is excreted in urine.

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 a correct adrenal function.

This test is very useful to estimate the real adrenal function, since free cortisol 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 OF THE METHOD

The Urinary Cortisol ELISA is a competitive enzyme immunometric assay (ELISA) where 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 the 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 blue colour that changes into yellow when the Stop Solution (H_2SO_4) is added. The colour intensity is inversely proportional to the cortisol concentration in the sample.

Cortisol concentration in the sample is calculated through a calibration curve.

4. REAGENTS, MATERIALS AND INSTRUMENTATION

4.1. Reagents and materials supplied in the kit

1. Calibrators (5 vials, CAL 0: 4 mL, CAL 1-4: 1 mL each;) ProClin >0.0015%, BSA 0.01%

CAL0	REF DCE002/1806-0
CAL1	REF DCE002/1807-0
CAL2	REF DCE002/1808-0
CAL3	REF DCE002/1809-0
CAL4	REF DCE002/1810-0

2. Control (2 vials, 1 mL each)
Concentration of Control is indicated on the Certificate of Analysis. ProClin >0.0015%, BSA 0.01%

Low control	REF DCE045/1801-0
High control	REF DCE045/1802-0

3. Conjugate (1 vial, 33 mL)
Cortisol conjugated with horseradish peroxidase (HRP). ProClin >0.0015%, BSA 0.1% REF DCE002/1802-0

4. Coated Microplate (1 microplate breakable)
Anti- Cortisol antibodies adsorbed on microplate REF DCE002/1803-0

5. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB (0.26 g/L) *(avoid any skin contact)*
ProClin <0.0015% **REF DCE004-0**

6. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15M *(avoid any skin contact)*
REF DCE005-0

7. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0.2M pH 7.4. ProClin >0.0015%
REF DCE054-0

4.2. Materials required but not provided
Distilled water

4.3. Auxiliary materials and instrumentation
Automatic dispenser
Precision Pipetting Devices
Microplate reader (450 nm, 620-630 nm)

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.
- ⚠ Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents (calibrators, controls, conjugate and wash solution) contain small amounts of ProClin™ 300 (>0.0015%, <0.06%) as preservative. Avoid contact with skin or mucosa.
- Classification according to Regulation (EC) No. 1272/2008 [CLP]

  Skin sensitivity, Category 1

  Warning

  Contains: ProClin 300

  Hazard statements:
  H317 - May cause an allergic skin reaction.

  Precautionary statements:
  P261 - Avoid breathing dust / fume / gas / mist / vapours / spray.
  P280 - Wear protective gloves/ protective clothing / eye protection / face protection / hearing protection.
  P321 - Specific treatment (see supplemental first aid instruction on this label).
  P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
  P362+P364 - Take off contaminated clothing and wash it before reuse.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous, 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 direct sunlight, metals or oxidants. Do not freeze the solution.

6. PRECAUTIONS
- 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.
- 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 validated for its intended use/purpose.
- 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 controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemic, icteric or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Fresh disposable tips must be used when pipetting assay reagents including samples, calibrators and controls to mitigate the risk of carryover contamination. Failure to do so may lead to invalid results.

7. REAGENT STORAGE AND STABILITY
Store the kit at 2 – 8°C in the dark.
- The kit is stable at 2 – 8°C until the expiry date stated on the external kit label.
- Once opened, the calibrators are stable at 2 – 8°C for 6 months.
- Once opened, the conjugate is at 2 – 8°C for 6 months.
- The diluted wash solution is stable for 30 days at 2 – 8°C.

Important note: open the bag containing the Coated Microplate only when it is at room temperature and close it immediately after use.

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8. SAMPLE COLLECTION AND STORAGE

The assay should be performed using urine samples.

Sample Storage	Duration
-20°C	< 6 months

9. PROCEDURE

9.1. Preparation of Calibrators and Controls

Before use, mix for 5 minutes with a rotating mixer.
The calibrators are ready for use and have the following concentration:

	C_0	C_1	C_2	C_3	C_4
ng/mL	0	1	5	30	200

The controls are ready to use; the concentration is printed on the label.

9.2. Preparation of the Conjugate

The conjugate is ready to use.

9.3. Preparation of the Wash Solution

Dilute the content of the vial "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.

It is possible to observe the presence of crystals within the concentrated wash solution; 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 also to transfer crystals completely by rinsing of the bottle, then mix until crystals are completely dissolved.

9.4. Preparation of Samples

The determination of cortisol can be performed in human 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-hour should be collected and mixed in a single container.

Store the sample at -20°C if the determination is not performed on the same day of the sample collection. Before using, mix gently, for 5 minutes, with a roller mixer.

9.5. Procedure

- **Allow all reagents to reach room temperature (22 – 28 °C) for at least 30 minutes.** At the end of the assay, immediately store the reagents at 2 – 8°C: avoiding 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 (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Control	Blank
Calibrator C_0-C_4	**10 µL**		
Sample/ Control		**10 µL**	
Conjugate	**300 µL**	**300 µL**	

Incubate 1 h at +37°C ±0.5°C.
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: if you use automated equipment, wash the wells at least 6 times.

TMB Substrate	**100 µL**	**100 µL**	**100 µL**

Incubate at 22 – 28°C for 15 minutes in the dark.

Stop Solution	**100 µL**	**100 µL**	**100 µL**

Shake gently the microplate.
Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.

10. QUALITY CONTROL

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples, and the results analysed with appropriate statistical methods.

The kit controls provided in the kit should be tested as unknowns and are intended to assist in assessing the validity of results obtained with each assay plate.

The mean concentration of each control level is documented in the QC report included with each kit. These mean concentration levels are determined over several assays which are run in duplicate in multiple locations across each plate.

DiaMetra recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This

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information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.

When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[6].

11. CALCULATION OF RESULTS

A variety of data reduction software packages are available, which may be employed to generate the mean calibration curve and to calculate the mean concentrations of unknown samples and controls. A 4-parameter logistic (4PL) curve fit, **including Calibrator 0 is required.** Other curve fitting algorithms are not recommended.

Alternatively, a calibration curve may be prepared on semi-log graph paper by plotting mean absorbance on the Y-axis against concentration of analyte on the X-axis. Calibrator 0 should be included in the calibration curve. Read the mean absorbance value of each unknown sample off the curve.

In order for the assay results to be considered valid the kit calibrators and control must fall within the specifications detailed in the lot specific certificate of analysis.

If a control is out of its specified range, the associated test results are invalid and samples must be retested.

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 24h/ 1000 = µg Cortisol/24h

12. EXPECTED VALUES

To determine the normal range for urine samples, 128 apparently healthy male and female adults were tested.

	n	Normal range urine (24h)
Adults	128	1.5 – 63 µg/24h

13. PERFORMANCE CHARACTERISTICS

Representative performance data are shown. Results obtained at individual laboratories may vary.

13.1. Analytical sensitivity

Analytical sensitivity was investigated through the LOB (white limit), the LOD (detection limit), the LOQ (quantification limit) and the anal sensitivity (A.S.).
The following table shows the criteria of the study and the results obtained.

	Criteria	Result (ng/mL)
LoB	60 replicates of Cal 0. used as "Blank". have been investigated in 5 different sessions over 3 days	0.28
LoD	6 urine samples with low cortisol concentration have been investigate over 10 assays in duplicate. performed in 5 days	0.47
LoQ	6 urine samples with low cortisol concentration have been investigate over 10 assays in duplicate. performed in 5 days	0.56
AS	20 replicates of Cal 0 and 5 replicates of Cal 1 have been assayed. A.S. has been calculated by linear regression	0.22

13.2. Precision and reproducibility (complex precision)

Precision and reproducibility have been assessed through 6 different urine samples with different concentration of Cortisol.
The table below shows the Within Run and Total CV%.

Sample	n°	Mean (ng/mL)	Within Run CV%	Total CV%
PS2	20	112.141	6.6%	12%
PS4	20	64.563	8.1%	12%
CT High	20	50.577	7.3%	11%
PS5	20	25.878	7.6%	10%
PS6	20	9.269	7.6%	11%
CT Low	20	3.438	7.0%	9%

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13.3. Analytical specificity

13.3.1. Interfering substances

Interference for Albumin. Acetylsalicylic Acid. Ibuprofen and Ascorbic Acid were studied by adding the interfering substance to the urine sample with a low and high Cortisol concentration. and by comparing its concentration to the unspiked sample.

The interference has been evaluated as "significant" if it causes a concentration bias >10% between spiked and unspiked sample.

The following table shows the results obtained:

Substance	Concentration	Interference
Albumin	5 mg/dL	No
Acetylsalicylic acid	3.62 mmol/L	No
Ibuprofen	2.42 mmol/L	No
Ascorbic Acid	5 mg/L	No

Conclusion: no interference has been found for Albumin. Acetylsalicylic Acid. Ibuprofen and Ascorbic Acid.

13.3.2. Cross-reactivity

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

Reagent	Cross-reactivity
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-sulphate	< 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 %

13.4. Correlation

137 urine samples were tested with the Urinary Cortisol ELISA kit and with a LC-MS method (reference)
The linear regression curve is:

n	Slope	Intercept (ng/mL)	R^2
137	1.008	-0.5019	0.83

14. LIMITATIONS OF USE

- As in the case of any diagnostic procedure. results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
- The performance characteristics of this assay have not been established in a paediatric population.

15. WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

All materials that have come into contact with samples and reagents must be disposed of in accordance with country. state and local regulations.

16. BIBLIOGRAPHY

1. Foster, L. B. and Dunn, R. T. Clin. Chem. 20/3 365(1974)
2. De Lacerda, et al J. Clin. Endocr. and Metab. 36,227 (1973)
3. Rolleri, E., et al Clin. Chim. Acta 66 319 (1976)
4. Kobayashi, Y., et al Steroids, 32 no 1(1978)
5. Akarawa, et al Anal. Biochem. 97 248 (1979)
6. Basic QC Practices On-line Course; http://www.Westgard.com.

17. REVISION IDENTIFIER

Additions or changes to the IFU are indicated by grey highlighting.

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18. PRODUCT COMPLAINTS AND TECHNICAL SUPPORT

For a patient/user/third party in the European Union and in countries with similar regulatory regime (Regulation 2017/746/EU on IVD Medical Devices); if. during the use of this device or as a result of its use. a serious incident has occurred. please report it to the manufacturer and/or its authorised representative and to your national regulatory authority.

The manufacturer can be contacted through their customer service or technical support team. The contact details can be found below and on the company website: www.diametra.com.

Ed. 03/2024 **DCM018-14**

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DiaMetra	Packaging Information Sheet	Mod. PIS000-2

IVD	DE	*In vitro* Diagnostikum		DE	Hergestellt von
	ES	Producto sanitario para diagnóstico *In vitro*		ES	Elaborado por
	FR	Dispositif medical de diagnostic *in vitro*		FR	Fabriqué par
	EN	*In vitro* Diagnostic Medical Device		EN	Manufacturer
	IT	Dispositivo medico-diagnostico *in vitro*		IT	Produttore
	PT	Dispositivos medicos de diagnostico *in vitro*		PT	Produzido por
	DE	Achtung, Begleitdokumente	yyyy-mm	DE	Herstellungs datum
	ES	Precaución, consulte los documentos adjuntos		ES	Fecha de fabricacion
	FR	Attention, veuillez consulter les documents d'accompagnement		FR	Date de fabrication
	EN	Caution, consult accompanying documents		EN	Date of manufacture
	IT	Attenzione, consultare la documentazione allegata		IT	Data di produzione
	PT	Atenção,consultar os documentos de acompanhamento		PT	Data de produção
yyyy-mm-dd	DE	Verwendbar bis		DE	Biogefährdung
	ES	Establa hasta (usar antes de último día del mes)		ES	Riesco biológico
	FR	Utiliser avant (dernier jour du mois indiqué)		FR	Risque biologique
	EN	Use by (last day of the month)		EN	Biological risk
	IT	Utilizzare prima del (ultimo giorno del mese)		IT	Rischio biologico
	PT	Utilizar (antes ultimo dia do mês)		PT	Risco biológico
	DE	Gebrauchsanweisung beachten	LOT	DE	Chargenbezeichnung
	ES	Consultar las instrucciones		ES	Codigo de lote
	FR	Consulter le mode d'emploi		FR	Numero de lot
	EN	Consult instructions for use		EN	Batch code
	IT	Consultare le istruzioni per l'uso		IT	Codice del lotto
	PT	Consultar instruções para uso		PT	Codigo do lote
Σ = xx	DE	Ausreichend für "n" Tests	CONT	DE	Inhalt
	ES	Contenido suficiente para "n" tests		ES	Contenido del estuche
	FR	Contenu suffisant pour "n" tests		FR	Contenu du coffret
	EN	Contains sufficient for "n" tests		EN	Contents of kit
	IT	Contenuto sufficiente per "n" saggi		IT	Contenuto del kit
	PT	Contém o suficiente para "n" testes		PT	Conteúdo do kit
	DE	Temperaturbereich	REF	DE	Bestellnummer
	ES	Límitaciôn de temperatura		ES	Nûmero de catálogo
	FR	Limites de température de conservation		FR	Réferéncès du catalogue
	EN	Temperature limitation		EN	Catalogue number
	IT	Limiti di temperatura		IT	Numero di Catalogo
	PT	Temperaturas limites de conservação		PT	Número do catálogo
	DE	Vor direkter sonneneinstrahlung schützen			
	ES	Mantener alejado de la luz solar			
	FR	Tenir à l'écart de la lumière du soleil			
	EN	Keep away from sunlight			
	IT	Tenere lontano dalla luce solare			
	PT	Mantenha longe da luz solar			

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