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DCM001-10
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CORTISOL ELISA

for routine analysis

Direct immunoenzymatic determination of Cortisol in human serum or plasma

IVD

LOT
See external label

2°C 8°C

Σ = 96 tests

REF DKO001

INTENDED USE

Eagle Biosciences Cortisol ELISA Assay Kit is a competitive immunoenzymatic colorimetric method for quantitative determination of Cortisol concentration in human serum or plasma. Cortisol ELISA Assay Kit is intended for research use only and is not intended for diagnostic procedures.

1. 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.

The amount of cortisol present in the serum undergoes diurnal variation, with the highest levels present in the early morning, and lower levels in the evening, several hours after the onset of sleep. Highest levels are at about 6-8 a.m. and lowest levels are at about midnight. 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.

2. PRINCIPLE OF THE METHOD

In the Cortisol ELISA Assay Kit, he 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 blue color that changes into yellow when the Stop Solution (H_2SO_4) is added.

The color intensity is inversely proportional to the Cortisol concentration of in the sample. Cortisol concentration in the sample is calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Cortisol Calibrators (5 vials, 1 mL each)

CAL0	REF DCE002/0106-0
CAL1	REF DCE002/0107-0
CAL2	REF DCE002/0108-0
CAL3	REF DCE002/0109-0
CAL4	REF DCE002/0110-0

2. Cortisol Control (1 vial, 1 mL)
See Control concentration on the Certificate of Analysis — REF DCE045/0103-0

3. Conjugate (1 vial, 21 mL)
Cortisol conjugated with Horseradish peroxidase (HRP) — REF DCE002/0102-0

4. Coated Microplate
(1 breakable microplate coated with anti-Cortisol antibodies) — REF DCE002/0103-0

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

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

7. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0.2M, Proclin < 0.0015% — REF DCE054-0

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

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

Notes

Store all reagents at 2-8°C in the dark.

Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable up to expiry date of the kit.

4. WARNINGS

- This Cortisol ELISA Assay Kit is intended for research use and 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 of the Cortisol ELISA Assay Kit contain small amounts of Proclin 300^{R} 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 Cortisol ELISA Assay Kit method allows the determination of Cortisol from 10 ng/mL to 500 ng/mL.

5. 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 of the Cortisol ELISA Assay Kit 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 Cortisol ELISA Assay Kit 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.
- 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 lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibration (C_0…C_4)

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

	C_0	C_1	C_2	C_3	C_4
ng/mL	0	10	50	150	500

The Calibrators are stable until the expiry date printed on the label. Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of the conjugate

The conjugate is ready to use. Mix gently, for 5 minutes, with a rotating mixer.

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

6.3. Preparation of the Sample

The determination of Cortisol with this kit can be performed in human plasma as well as in serum.

Store the sample at -20°C if the determination is not performed on the same day of the sample connection. Avoid repetitive freezing and thawing of samples.

The Control is ready for use.

6.4. 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.

6.5. Procedure

- **Allow all reagents to reach room temperature (22-28°C).** 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 (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	**20 µL**		
Sample/ Control		**20 µL**	
Conjugate	**200 µL**	**200 µL**	
Incubate 1 hour at 37°C. Remove the contents from each well. Wash the wells 3 times with 300 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 15 minutes in the dark at room temperature (22÷28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of 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 calibration 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.

8. RESULTS

8.1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the calibration curve (C_0-C_4) and of each sample.

8.2. Calibration Curve

Plot the mean value of absorbance (Em) of the calibrators (C_0-C_4) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

8.3. 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.

9. REFERENCE VALUES

The serum or plasma Cortisol reference values are:

60 - 230 ng/mL beetwen 8.00 – 10.00 A.M.
30 – 150 ng/mL at 4.00 P.M.

Subject treated with ACTH: 280 - 600 ng/mL
Subject treated with dexamethasone: 0 - 50 ng/mL

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra Assay

Within run variation was determined by replicate (20x) the measurement of three different sera in one assay. The within assay variability is ≤ 9.0%.

10.1.2. Inter Assay

Between run variation was determined by replicate (10x) the measurement of three different sera in different lots. The between assay variability is ≤ 9.8%.

10.2. Specificity

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 %

10.3. Accuracy

The recovery of 12.5 – 25 – 50 – 100 ng/mL of Cortisol added to samples gave an average value (±SD) of 103.56% ± 8.17% with reference to the original concentrations.

10.4. Sensitivity

The lowest detectable concentration of cortisol that can be distinguished from the Calibrator zero is 2.44 ng/mL at the 95% confidence limit.

10.5. Correlation

The new Diametra Cortisol ELISA kit was compared to a chemiluminescenze Cortisol kit commercially available. 19 serum samples were analysed.
The linear regression curve was calculated:
Y = 1.30*X - 61.96
r^2 = 0.900

The new Diametra Cortisol ELISA kit was compared to the old Diametra Cortisol ELISA kit. 60 serum samples were analysed.
The linear regression curve was calculated:
Y = 0.88*X + 15.71
r^2 = 0.933

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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