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	See external label	2°C-8°C	Σ=96 tests	REF Cat # 6101Z

Cortisol ELISA

Cat # 6101Z

Test	Cortisol ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Indirect ELISA: Antigen Coated Plate
Detection Range	0-500 ng/ml
Sample	20ul serum
Specificity	100%
Sensitivity	5 ng/ml
Total Time	~ 80 min
Shelf Life	12-14 months

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Cortisol concentration in serum and plasma.

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.

PRINCIPLE

Cortisol (antigen) in the sample competes with horseradish peroxidase-Cortisol (enzyme-labelled antigen) for binding onto the limited number of anti-Cortisol (antibody) sites on the microplates (solid phase).
After incubation, the bound/free separation is performed by a simple solid-phase washing.
The enzyme substrate (H_2O_2) and the TMB-Substrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbencies are determined. Cortisol concentration in the sample is calculated based on a series by a set of standard.
The colour intensity is inversely proportional to the Cortisol concentration in the sample.

REAGENT, MATERIAL AND INSTRUMENTATION

Reagent and material supplied in the kit

1. Cortisol Standards 5x (1 vial = 1 mL)
   STD0 REF **DAS0/6101Z**
   STD1 REF **DAS1/6101Z**
   STD2 REF **DAS2/6101Z**
   STD3 REF **DAS3/6101Z**
   STD4 REF **DAS4/6101Z**

2. Control (1vial = 1.0 mL) REF **DACON/6101Z**

3. Conjugate (1 bottle) 21.0 mL
   Cortisol-HRP conjugate REF **DA-C/6101Z**

4. Coated Microplate
   Anti-Cortisol-IgG adsorbed on microplate
   (1 microplate breakable) REF **DA-P/6101Z**

5. TMB-substrate (1 bottle) 15 mL
H_2O_2-TMB 0.25gr/L
(avoid any skin contact) **REF DA-T/6101Z**

6. Stop solution (1 bottle) 15 mL
Sulphuric acid 0.15 mol/L
(avoid any skin contact) **REF DA-S/6101Z**

Reagents necessary not supplied
Distilled water.

Auxiliary materials and instrumentation
Automatic dispenser.
Microplates reader

Notes
Store all reagents between +2 and + 8C°in the dark.Open the bag of reagent 3 (Antibody) only when it is at room temperature and close immediately after use.
Do not remove the adhesive sheets on the unused strips .The kit once is open, it is stable up to expiration date.

PRECAUTION

- Do not use heavily haemolysed samples.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Avoid the exposure of reagent TMB/H2O2 to directed sunlight, metals or oxidants.
- 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 synthetic steroids.

PROCEDURE

1. Preparation of the Standard (S_0,S_1,S_2,S_3,S_4) and control
The standard has the following concentration of Cortisol:

	S_0	S_1	S_2	S_3	S_4
ng/ml	0	10	50	150	500

Stability: until the expiration date printed on the kit.
Once open are stable for six months at +4°C.

2. Preparation of the Sample
The determination of Cortisol can be performed in 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.

3. PROCEDURE
As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S_0-S_4), two for each sample and one for Blank.

Reagent	Standard	Sample	Blank
Standard S0-S4	**20 µL**		
Control	**20 µL**		
Samples	**20 µL**		
Conjugate	**200 µL**	**200 µL**	
Incubate 1 hour at 37 °C. Remove the contents from each well; wash the wells with 300 µL of distilled water. Repeat the washing procedure by draining the water completely.			
TMB substrate	**100 µL**	**100 µL**	**100 µL**
Incubate 15 minutes in the dark at room temperature (20-25°C).			
Stop solution	**100 µL**	**100 µL**	**100 µL**
Read the absorbance (E) at 450 nm against Blank			

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 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.

LIMITATION OF PROCEDURE

1. Assay Performance.

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemeic or haemolysed specimen(s) should similarly not be used. 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 one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

2. Interpretation.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

RESULTS

1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample

2. Standard Curve

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

3. Calculation of Results

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

4. Range of Control:

Please refer to the range information from the COA for the given Lot number. The obtained value for the control should fall within the specified range.

REFERENCE VALUE

The serum or plasma Cortisol reference value are:

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

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

PERFORMANCE AND CHARACTERISTICS

1. Intra Assay Variation

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 7%.

2. Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 9.32%.

3. Specificity

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

Cortisol	100 %
Cortisone	10.8 %
11α deoxycortisol	18.7 %
Corticosterone	2.4 %
Progesterone	0.1 %
Aldoosterone	$1x10^{-2}$ %
11α OH Progesterone	$1x10^{-2}$ %
Cholesterol	< $1x10^{-6}$ %

4. Accuracy
The recovery of 50 – 100 – 200 – 400 ng/mL of Cortisol added to samples gave an average value (±SD) of 101.2% ± 6.65% with reference to the original concentrations.

5. Sensitivity
The lowest detectable concentration of cortisol that can be distinguished from the zero standard is 5 ng/ml at the 95 % confidence limit.

6. Correlation with RIA
The DAI Cortisol ELISA was compared to another commercially available Cortisol assay. Serum samples of 33 females and 30 males were analysed according in both test systems.
The linear regression curve was calculated
$y = 0.944\,x + 3.2$
$r = 0.98$ ($r^2 = 0.96$)

8. Hook Effect
The Cortisol ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 1000 ng/ml

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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