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Labor Diagnostika Nord GmbH & Co. KG

Instructions for use

Cortisol Urine ELISA

REF **MS E-5100**

96

+2 +8 °C

IVD

CE

Cortisol Urine ELISA

INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Free Cortisol concentration in Urine

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 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 a correct surrenic functionality.
This test is very useful to estimate the real surrenic function, because is dose 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.

PRINCIPLE

Urinary 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-Sustrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbances are determined.
Urinary Cortisol concentration in the sample is calculated based on a series of standard.
The colour intensity is inversely proportional to the urinary cortisol concentration in the sample.

Reagent, material and instrumentation

Reagent and material supplied in the kit

Standards

	Cat. no.	Standard	Concentration	Volume/Vial
STANDARD A	**MS E-5101**	Standard 0	0 ng/ml	1 ml
STANDARD B	**MS E-5102**	Standard 1	10 ng/ml	1 ml
STANDARD C	**MS E-5103**	Standard 2	50 ng/ml	1 ml
STANDARD D	**MS E-5104**	Standard 3	150 ng/ml	1 ml
STANDARD E	**MS E-5105**	Standard 4	500 ng/ml	1 ml

SD0 – STD4, 5x (1 vial = 1 mL)

INC-BUFF **MS E-5113 Incubation buffer**

(1 bottle) 100 mL; Phosphate buffer 50 mM pH 7.4; BSA 1 g/L

CONJUGATE-CONC **MS E-5140 Conjugate**

(1 bottle) 1 mL; Cortisol-HRP conjugate

96 **MS E-5131 Coated Microplate**

(1 microplate breakable); Anti-Cortisol-IgG adsorbed on microplate

SUBSTRATE **MS E-0055 TMB-substrate**

(1 bottle) 15 mL; H_2O_2-TMB 0.26 g/L (avoid any skin contact)

STOP-SOLN **MS E-0080 Stop solution**

(1 bottle) 15 mL; Sulphuric acid 0.15 mol/L (avoid any skin contact)

CONTROL 1 **MS E-5151 Low Control**

(1 vial = 1 mL); Ready to use

CONTROL 2 **MS E-5152 High Control**

(1 vial = 1 mL); Ready to use

Reagents necessary not supplied

Distilled water

Auxiliary materials and instrumentation

Automatic dispenser
Microplates reader

Note

Store all reagents at 2 °C – 8 °C in the dark.
Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close immediately after use.
Do not remove the adhesive sheet from the unused strips.

PRECAUTION

The reagent contains Proclin 300 as preservative.
Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants.
Maximum precision is required for reconstitution and dispensation of the reagents.
Do not use different lots of reagents.
Do not use heavily haemolized samples.
This method allows the determination of Cortisol from10 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.

PROCEDURE

Preparation of the Standard (S_0, S_1, S_2, S_3, S_4)

The standards have the following concentration of Cortisol:

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

Stable when stored at +4 °C until the expiration date of the kit;
once opened the standards are stable six months at +4 °C.

Preparation of diluted Conjugate

Prepare immediately before use.
Add 10 µL Conjugate (reagent 3) to 1.0 mL of Incubation Buffer (reagent 2). Mix gently.
Stable for 3 hours at room temperature.

Preparation of 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 °C – 8 °C or at – 20°C for periods longer than a week.
Dilute the urine sample (1 + 1) with Incubation buffer (e.g. 100 µL + 100 µL)

Procedure

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

Reagent	Standard/Control	Samples	Blank
Standard S_0-S_4 Controls	10 µL		
Diluted samples		10 µL	
Diluted Conjugate	300 µL	300 µL	
Incubate at 37°C for *1 hour*. Remove the contents from each well; wash the wells with 400 µL of distilled water. Repeat the washing procedure by draining the water completely			
TMB substrate	100 µL	100 µL	
Incubate at room temperature 22÷28°C for *15 minutes* in the dark			
Stop solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank.			

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.

LIMITATION OF PROCEDURE

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.

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

Mean Absorbance

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

Standard Curve

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

Calculation of Results

As the standards are prediluted, the dilution factor 2 for the urine samples has not to be taken into account for the calculation. The concentration of the samples (in ng/mL) can be read directly from the standard curve. Interpolate the values of the samples on the standard 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/ 1.000 = µg Cortisol/24h

Reference Value

50 – 190 µg/24 hours

Performance and Characteristics

Precision

Intra-Assay Variation

Within run variation was determined by replicate measurements (10x) of three different control sera in one assay. The within assay variability is ≤7%.

Inter-Assay Variation

Between run variation was determined by replicate measurements (10x) of three different control sera in different lots of kit. The between assay variability is ≤9%.

Accuracy

The recovery of 25 - 50 - 100 - 200 ng/mL of Urinary Cortisol added to a sample gave an average value (±SD) of 101.89% ± 4.55% with reference to the original concentrations.

Sensitivity

The lowest detectable concentration of urinary cortisol that can be distinguished from the zero standard is 2.0 ng/ml at the 95 % confidence limit.

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%
Aldosterone	$1x10^{-2}$%
11α OH Progesterone	$1x10^{-2}$%
Cholesterol	< $1x10^{-6}$%

Correlation with RIA

The Urinary Cortisol ELISA was compared to another commercially available Urinary Cortisol assay. 50 serum samples were analysed according in both test systems.
The linear regression curve was calculated:
$y = 1.10\ x - 1.56$
$r^2 = 0.949$

y = Urinary Cortisol ELISA
x = Cortisol Immunotech RIA

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

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TROUBLESHOOTING

ERRORS / POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed)

too high within-run CV%

- reagents and/or strips not pre-warmed to room temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run CV %

- incubation conditions not constant (time, temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)

person-related variation

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!