
Instructions for use
Cortisol Urine ELISA

REF

MS E-5100

96

+2  **+8**
°C

IVD

CE

URINARY FREE CORTISOL ELISA

1. PRINCIPLE AND INTENDED USE

Enzymeimmunoassay for the quantitative determination of free cortisol in urine. For in-vitro diagnostic use only. Cortisol (antigen) in the sample competes with horseradish peroxidase-Cortisol (enzyme-labeled 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 Conjugate (H₂O₂) and the TMB Substrate are added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbance are determined. Cortisol concentration in the sample is calculated based on a series of standard. The color intensity is inversely proportional to the Cortisol concentration in the sample.

2. REAGENT, MATERIAL AND INSTRUMENTATION

2.1 Reagent and material supplied in the kit

STANDARD A	
STANDARD B	
STANDARD C	
STANDARD D	
STANDARD E	Cortisol Standards, 1 ml
CONTROL 1	
CONTROL 2	Cortisol Controls, 1 ml
INC-BUFF	Incubation buffer (1 bottle), 100 ml, Phosphate buffer 50 mM pH 7.4; BSA 1 gr/L
CONJUGATE-CONC	Conjugate (1 bottle), 0.4 ml, Cortisol-HRP conjugate
96	Microplate, Anti-Cortisol-IgG adsorbed on microplate
SUBSTRATE	TMB Substrate (1 bottle), 12 ml, H ₂ O ₂ .TMB 0.25gr/L (avoid any skin contact)
STOP-SOLN	Stop Solution (1 bottle), 12 ml, Sulphuric acid 2 mol/L (corrosive:avoid any skin contact)

2.2 Notes

Store all reagents between +2 and + 8°C in the dark.
Open the bag of Microplate only when it is at room temperature and close immediately after use.
Do not remove the adhesive sheets on the strips until used

2.3 Reagents necessary which are not supplied with the kit

Distilled water.

2.4 Auxiliary materials and instrumentation

Disposable glass tubes.
Automatic dispenser.
Microplates reader

3. PRECAUTION

- Maximum precision is required for reconstitution and dispensation of the reagents.
- Avoid the exposure of reagent TMB/H₂O₂ to direct 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.

4. PROCEDURE

4.1 Preparation of the Standards

The standard has the following concentration of Cortisol:

	S _A	S _B	S _C	S _D	S _E
ng/ml	0	10	50	150	500

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

4.2 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**

4.3 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 $2 \pm 8^\circ\text{C}$ or at -20°C for period longer than a week.

Dilute the urine sample (1 + 1) with Incubation buffer (e.g. 100 μL + 100 μL)

4.4 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	Samples	Blank
Standards ($S_A - S_E$) + 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	100 μL
Incubate at room temperature $22 \pm 28^\circ\text{C}$ for 15 minutes in the dark			
Stop solution	100 μL	100 μL	100 μL
Read the absorbance (E) at 450 nm against Blank..			

5. 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.

6. LIMITATION OF PROCEDURE

a. Assay Performance

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic 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.

b. 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.

7. RESULTS

a. Mean Absorbance

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

b. Standard Curve

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

c. Calculation of Results

As the standards are prediluted, the dilution factor 2 for the urinesamples 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:

$$\text{ng/mL} \times \text{Vol(mL) urine 24 h} / 1.000 = \mu\text{g Cortisol/24h}$$

8. REFERENCE VALUE

50 – 190 µg/24 hours

9. PERFORMANCE AND CHARACTERISTICS

Precision

a. 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 7.4%.

b. 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 7.8%.

c. Specificity

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

Cortisol	100%
Cortisone	10.8%
11 α-deoxycortisol	18.7%
Corticosterone	2.4%
Progesterone	0.1%
Aldosterone	1x10 ⁻² %
11a OH Progesterone	1x10 ⁻² %
Cholesterol	< 1x10 ⁻⁶ %

d. Accuracy

The recovery of 50 – 100 – 200 – 400 ng/mL of Urinary Cortisol added to a sample gave an average value (±SD) of 105% ± 7.1% with reference to the original concentrations.

e. 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.

f. Correlation with RIA

The Urinary Cortisol ELISA was compared to another commercially available Urinary Cortisol assay. Serum samples of 23 females and 27 males were analysed according in both test systems.

The linear regression curve was calculated

$$y = 1.1x - 1.6$$

$$r = 0.976 \quad (r^2 = 0.948)$$

g. Hook Effect

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













10. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

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5. Akarawa, et al Anal. Biochem. 97 248 (1979)

Symbols:

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