Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Cortisol (Serum/Urine) ELISA Kit

07/20

(Catalog # E4849-100; 96 assays, Store kit at -20°C)

I. Introduction:

Cortisol, also known as hydrocortisone, is a naturally occurring corticosteroid hormone produced by the adrenal gland. Cortisol secretion is regulated by the pituitary hormone called adrenocorticotropic hormone (ACTH) and typically shows a diurnal cycle with the highest levels in the early morning and lowest levels at night. Cortisol is released in response to stress and low blood-glucose concentration and it plays a key role in homeostatic maintenance. Cortisol can increase blood pressure and blood sugar levels, suppress the immune system, aid in the metabolism of fat, protein, and carbohydrates, and reduce bone and collagen formation. Abnormal cortisol production can cause a number of different problems. Excess cortisol can result in depressive disorder, certain forms of hypertension, stress and Cushing's disease, whereas insufficient cortisol can lead to nausea, diarrhea, joint pain, Addison's disease and Nelson's syndrome. In animals, cortisol is known as a stress hormone in response to physical and emotional stresses. Cortisol can be measured in blood, saliva and urine and its concentration generally ranges from 30-250 ng/ml in plasma and from 10-170 µg/day in urine. The concentration of cortisol in saliva and serum is also found to be correlated. BioVision's Cortisol (Serum/Urine) ELISA kit is a competitive enzyme immunoassay kit and its readout signal is inversely proportional to the cortisol concentration. The kit is simple, easy and fast to determine the cortisol concentration in different biological samples such as serum and urine. It can detect cortisol range between 5.6 and 450 ng/ml within 90 minutes (LOD 2 ng/ml).

II. Applications:

In vitro quantitative determination of cortisol
Detection Range: 5.6 – 450 ng/ml
Sensitivity: 2 ng/ml

III. Sample Type:

Serum and urine

IV. Kit Contents:

Components	E4849-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4849-100-1
Cortisol Standard	1 vial	Yellow	E4849-100-2
HRP Conjugate Stock	25 µl	Blue	E4849-100-3
Antibody	7 ml	NM/Red	E4849-100-4
TMB substrate	10 ml	Amber	E4849-100-5
Stop Solution	10 ml	NM/Blue	E4849-100-6
Wash Buffer (10X)	50 ml	NM	E4849-100-7
Sample Diluent	40 ml	NM/Red	E4849-100-8
Standard Buffer	20 ml	WM	E4849-100-9
Conjugate Buffer	7.5 ml	NM/Green	E4849-100-10
Plate Sealers	4	--	E4849-100-11

V. User Supplied Reagents and Equipment:

- Ethyl acetate
- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards and sample dilutions

VI. Storage and Handling:

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit is stable for 2 months at 4°C.

VII. Reagent and Standard Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Antibody, TMB Substrate, Stop Solution, Sample Diluent, Standard Buffer and Conjugate Buffer:** Ready to be used. After use, store at 4°C.
- **HRP Conjugate Stock**: Spin briefly before opening the tube. Pipet 10 µl of HRP Conjugate Stock into Conjugate Buffer (7.5 ml) bottle to prepare **conjugate working solution**. Vortex the bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.
- **Wash Buffer (10X)**: Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored at 4°C for one month.
- **Cortisol Standards**: Reconstitute the Cortisol standard by adding 1.5 ml of Standard Buffer to prepare 450 ng/ml standard (S5). Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely. Perform 3-fold serial dilutions starting from S5 (e.g. mix 0.5 ml standard with 1.0 ml of Standard Buffer) to prepare standards S4 to S1 sequentially. S0 is Standard Buffer. The standards are stable at -20°C for up to 3 weeks.

Standards	S0	S1	S2	S3	S4	S5
Concentrations (ng/ml)	0	5.6	16.7	50	150	450

VIII. Sample Preparation:

- **Urine**

1. Centrifuge 0.5 ml of urine at 10,000 x g for 5 min to remove any precipitate and transfer 200 µl of supernatant into a clean eppendorf tube.
2. Add 200 µl of ethyl acetate into the tube and vortex for 10 min.
3. Spin the sample using 10,000 g for 10 min. Collect the upper phase and transfer 50 µl into a clean eppendorf tube.
4. Incubate the sample in a 37°C incubator for 1 hr to evaporate ethyl acetate.
5. After the sample is totally dry, add 50 µl of water into the tube.
6. Dilute the sample 5-fold with Sample Diluent (For example, mix 50 µl with 200 µl of Sample Diluent).
7. Use 50 µl per well for the assay.

- **Serum**

1. Dilute serum 10-fold with Sample Diluent (For example, mix 30 µl of serum with 270 µl of Sample Diluent).
2. Use 50 µl per well for the assay.

IX. Cortisol ELISA Assay Protocol:

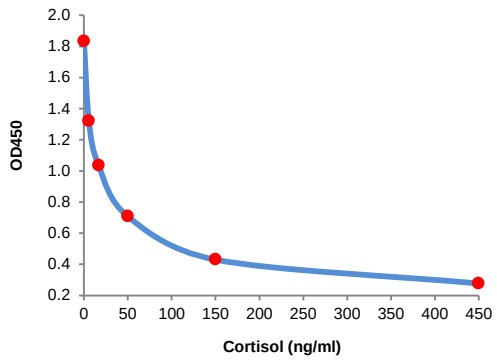
Notes: We recommended that all standards and samples are run in duplicate. A Standard curve must be run each time an assay is performed.

1. Prepare all reagents, standards and samples as sections VII and VIII specify respectively.
2. Add 50 µl of **Standards or Samples** per well. Then add 50 µl of **conjugate working solution** and 50 µl of **Antibody** to the above wells.
3. Cover the microtiter plate with plate sealer and mix well. Incubate the plate at room temperature (25°C) for 60 min.
4. Aspirate all reagents and wash each well 5 times: add 250 µl of **1X Wash Buffer** and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash. (Complete removal of wash buffer is essential for accurate results.) Repeat wash step 4 more times.
5. Add 100 µl of **TMB Substrate** to each well. Tap or shake the plate occasionally to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no cortisol (S0). When its reading is approximately 0.8, add 50 µl of **Stop Solution** and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm.

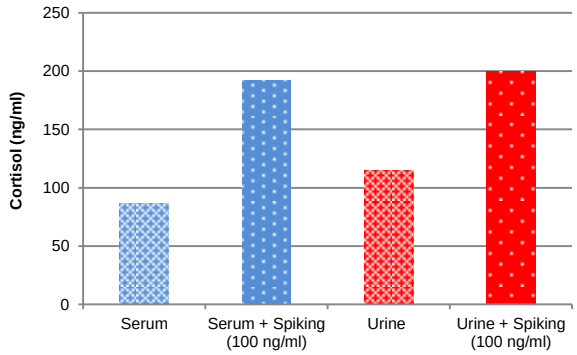
X. Calculation:

The Standard Curve is done by plotting the relative absorbance of the standards vs. cortisol concentrations. The cortisol concentration of each sample, which can be read from the calibration curve, is multiplied by the corresponding dilution factor.

A.



B.



Figures. **A.** Cortisol standard curve (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B.** Spike recovery experiment: Human serum and urine samples were spiked with cortisol (100 ng/ml) and showed 80-100% recovery.

XI. RELATED PRODUCTS:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315)
Folic Acid ELISA Kit (Cat. No. E4523)
Caffeine Acid ELISA Kit (Cat. No. E4558)
His-Tag Protein ELISA Kit (Cat. No. E4550)
DYKDDDDK-Tag Protein ELISA Kit (Cat. No. E4700)
Isoniazid ELISA Kit (Cat. No. E4765)
Mycophenolic Acid ELISA (Cat. No. E4819)
Ampicillin ELISA Kit (Cat. No. E4350)
Quinolone ELISA Kit (Cat. No. E4530)
Vancomycin ELISA Kit (Cat. No. E4605)
GST Tag ELISA Kit (Cat. No. E4690)
Bisphenol A ELISA Kit (Cat. No. E4722)
Carnosine ELISA Kit (Cat. No. E4766)

FOR RESEARCH USE ONLY! Not to be used on humans.