

Cortisol (human/mouse/rat) ELISA Kit

(Catalog # K7430-100, 100 assays; Refer to Section V for Storage)

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I. Introduction:

BioVision's Cortisol Enzyme-Linked Immunosorbent Assay (ELISA) Kit is an in vitro assay for the quantitative measurement of Cortisol in serum and plasma. Cortisol is a steroid hormone released from the adrenal cortex in response to the hormone ACTH. It increases blood pressure and blood sugar levels, and suppresses the immune system. Cortisol acts through specific intracellular receptors and has effects on numerous physiologic systems, including immune system, glucose-counter regulation, vascular tone, substrate utilization and bone metabolism. It exists in the blood as either a free form or bound to corticosteroid-binding globulin (CBG). The amount of Cortisol present in serum undergoes diurnal variation, with the highest levels present in the early morning and lower levels at night. BioVision's Cortisol ELISA kit is a competitive enzyme immunoassay kit. The assay employs an antibody specific for cortisol coated on a 96-well plate. Cortisol in a sample or a HRP molecule which has Cortisol covalently attached to it is bound to the wells by immobilized antibody in a competitive manner. After a simultaneous incubation, the excess reagents are washed and a TMB substrate is added to the wells. Color develops in proportion to the amount of bound Cortisol HRP conjugate, and the intensity is inversely proportional to the concentration of Cortisol in the sample. Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Sensitivity of the kit is 1 ng/ml and detection range is from 5 ng/ml to 480 ng/ml. The cross-reactivity of this ELISA assay is 6.8% for prednisolone, 4.22% for cortisone, and <0.1% for other hormones, including 11-deoxycortisol, estradiol, testosterone, and progesterone. The intra-assay reproducibility as measured by the coefficient of variation (CV) is < 8 % & inter-assay has CV < 12 %.

II. Application:

Quantitative measurement of Cortisol

III. Specificity:

Human, mouse, rat

IV. Sample Type:

- Serum and plasma
- Cell culture supernatant

V. Kit Contents:

Components	K7430-100	Cap Code	Part No.	Storage Temp.
Plate Coated with Cortisol Ab	12 stripsx8 wells	-	K7430-100-1	-20°C
Assay Diluent	15 ml	WM	K7430-100-2	4°C
Wash Buffer (10x)	20 ml	NM/Brown	K7430-100-3	4°C
Standard Diluent	15 ml	NM	K7430-100-4	4°C
Cortisol Standard (4800 ng/ml)	0.5 ml	Yellow	K7430-100-5	-20°C
Cortisol HRP Conjugate (2000x)	8 µl	Green	K7430-100-6	4°C
TMB Substrate	11 ml	Amber	K7430-100-7	4°C
Stop Solution	11 ml	NM/Blue	K7430-100-8	4°C
Plate Sealer	2	-	K7430-100-9	RT/4°C

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Distilled or deionized water.

VII. Storage Conditions and Reagent Preparation:

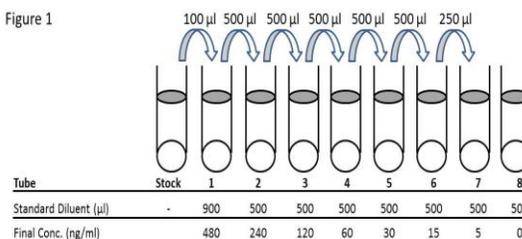
Kit can be used within one year if stored according to storage instructions. Avoid repeated freeze-thaw cycles. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

- **Wash Buffer (10x):** Dilute with deionized or distilled water to a final working 1x buffer concentration. If the Wash Buffer (10x) contains visible crystals, warm to room temperature and mix gently until dissolved before dilution.
- **Cortisol HRP Conjugate (2000x):** Dilute to 1x in Assay Diluent. Dilute only the necessary amount of Cortisol HRP Conjugate just before use. Recommendation: Dilute necessary amount of Cortisol HRP Conjugate 10 fold in 1x Wash Buffer. Further dilute this solution 200 fold in Assay Diluent to the 1x concentration.

VIII. Assay Protocol:

1. Bring all Buffers and desired number of Ab coated strips to room temperature (18 - 25°C) before use. It is recommended to run all Standard dilutions in duplicate.
2. Prepare a series of dilutions for Cortisol Standard (4800 ng/ml) in Standard Diluent as shown in Figure 1. Mix each tube gently and thoroughly before the next transfer. Standard Diluent alone serves as the zero Standard (0 ng/ml). **Note:** Discard unused diluted Standard solution.
3. Pipette 20 µl of Cortisol Standards or sample into their respective wells.
4. Add 100 µl of 1x Cortisol HRP Conjugate/well into appropriate wells. Cover

Figure 1



- wells with Plate Sealer and incubate with gentle shaking for 1-2 min. at room temperature. Incubate for 1 hr at 37°C.
- Discard the solution. Wash 4 times (each wash for 3-4 min.) with 200 μ l 1x Wash Buffer with gentle shaking.
 - Add 100 μ l of TMB Substrate/well and gently shake. Measure absorbance at 650 nm for 1-4 min. at room temperature to monitor the blue color development, intensity of which is inversely proportional to the concentration of cortisol in samples and Standards.

Notes:

- Incubation time after addition of TMB substrate can be optimized to avoid over-development of color. Recommended absorbance is ~0.8-1.2 at 650 nm.
 - Optional: Prepare one parallel well for background control and add TMB Substrate.
- Add 100 μ l of Stop Solution into each well including background control and mix with gentle shaking. Remove air bubbles if any. Read at 450 nm within 5 min.
 - Calculation:** Calculate the mean absorbance for each set of duplicate Standards. Plot Cortisol Standard Curve. Calculate Cortisol concentration of sample by interpolation of the Standard Curve. If sample was diluted, multiply the value by dilution factor to calculate the concentration of Cortisol in the sample.

Note: Background subtraction for each reading is optional for calculating the sample Cortisol concentration, and will not change the final results.

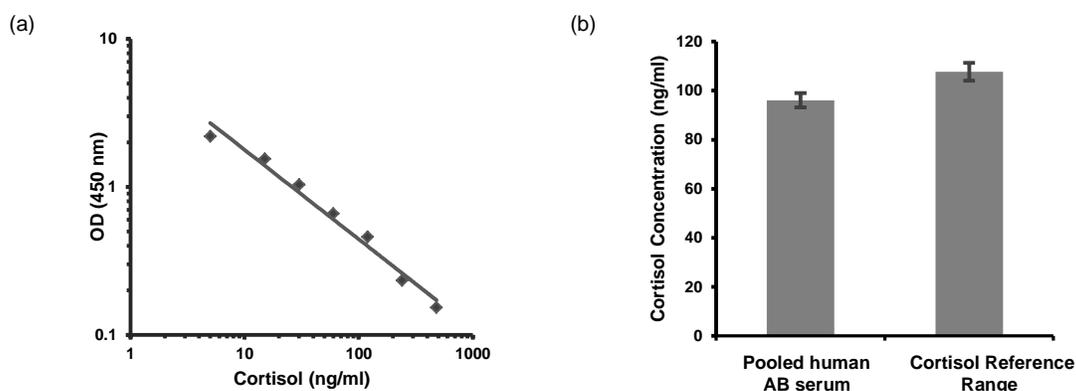


Figure 2: a) Cortisol Standard Curve. This standard curve is for demonstration only. A standard curve must be run with each assay. b) Measurement of Cortisol concentration in human serum. Sample of pooled human serum (20 μ l) was analyzed following the kit protocol (left) and compared to the literature values (right).

IX. RELATED PRODUCTS:

Adrenocorticotrophic Hormone (ACTH) (human) ELISA Kit (K4003-100)
 Prolactin (human) ELISA Kit (K4687-100)
 Prolactin (mouse/rat) ELISA Kit (K4688-100)

Growth Hormone (human) ELISA Kit (K7412-100)
 Luteinizing Hormone [LH] (human) ELISA Kit (K7426-100)
 Thyroid Stimulating Hormone (human) ELISA Kit (K7411-100)

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