

# Cortisol (Saliva) [human] ELISA Kit

(Catalog # K7434-100, 100 assays; Store at -20°C)

4/15

## I. Introduction:

BioVision's Cortisol (Saliva) Enzyme-Linked Immunosorbent Assay (ELISA) Kit is an in vitro assay for the quantitative measurement of Cortisol in saliva. 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). While only 1-10% of Cortisol in the blood exists in the unbound/free form, this fraction represents the active pool of Cortisol eliciting the biological response, and is capable of diffusing into target tissues and saliva. Studies consistently report high correlation between salivary and serum cortisol levels, indicating that salivary cortisol can reliably estimate serum Cortisol levels. BioVision's Cortisol (Saliva) 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 and HRP labeled Cortisol compete for binding to the immobilized antibody. After simultaneous incubation, the excess reagents are washed away 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 1 ng/ml to 100 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.

## II. Application:

Quantitative measurement of salivary Cortisol

## III. Specificity:

Human

## IV. Sample Type:

- Saliva

## V. Kit Contents:

Components	K7434-100	Cap Code	Part No.
Plate Coated with Cortisol Ab	12 stripsx8 wells	-	K7434-100-1
Assay Diluent	15 ml	WM	K7434-100-2
Wash Buffer (10x)	20 ml	NM/Brown	K7434-100-3
Standard Diluent	15 ml	NM	K7434-100-4
Cortisol Standard (4800 ng/ml)	0.5 ml	Yellow	K7434-100-5
Cortisol HRP Conjugate	1 Vial	Green	K7434-100-6
TMB Substrate	11 ml	Amber	K7434-100-7
Stop Solution	11 ml	NM/Blue	K7434-100-8
Plate Sealer	2	-	K7434-100-9

## 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:

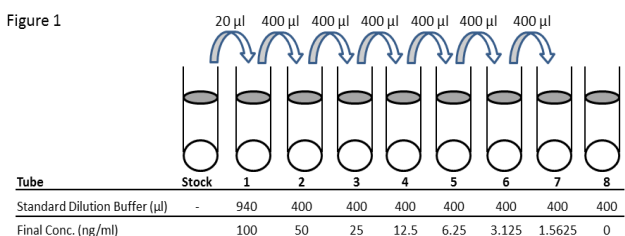
Kit can be used within one year if stored properly at -20°C. Avoid repeated freeze-thaw cycles. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Return unused wells to the pouch containing desiccant pack, reseal along the entire edge.

- **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:** Reconstitute with 16 µl sterile dH<sub>2</sub>O to make 1000x stock solution. Let the vial sit at room temperature for 10 min. Tap gently to mix, make sure it is completely dissolved. Store the reconstituted Cortisol-HRP Conjugate solution at 4°C. Use within two months.

## VIII. Assay Protocol:

1. **Sample Preparation:** Collect saliva sample and freeze/thaw at least once. Centrifuge at 3000xg, room temperature for 5 min. to remove the mucins (pellet) in sample. Collect clear supernatant for the assay.
2. 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.
3. 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

Figure 1





- the zero Standard (0 ng/ml). **Note:** Discard unused diluted Standard solution.
- Pipette 20  $\mu$ l of Cortisol Standards or sample into their respective wells.
  - Make 1x solution of Cortisol HRP Conjugate (1000x) using Assay Diluent just before use. Make as much as needed. Add 100  $\mu$ l of 1x Cortisol HRP Conjugate/well into appropriate wells. Cover wells with Plate Sealer and incubate with gentle shaking for 5 min. at room temperature. Incubate for 1 hr at 37°C.
  - Discard the solution. Wash 4 times (each time 3-4 min.) with 200  $\mu$ l 1x Wash Buffer with gentle shaking.
  - Add 100  $\mu$ 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 the sample and Standards.

**Notes:**

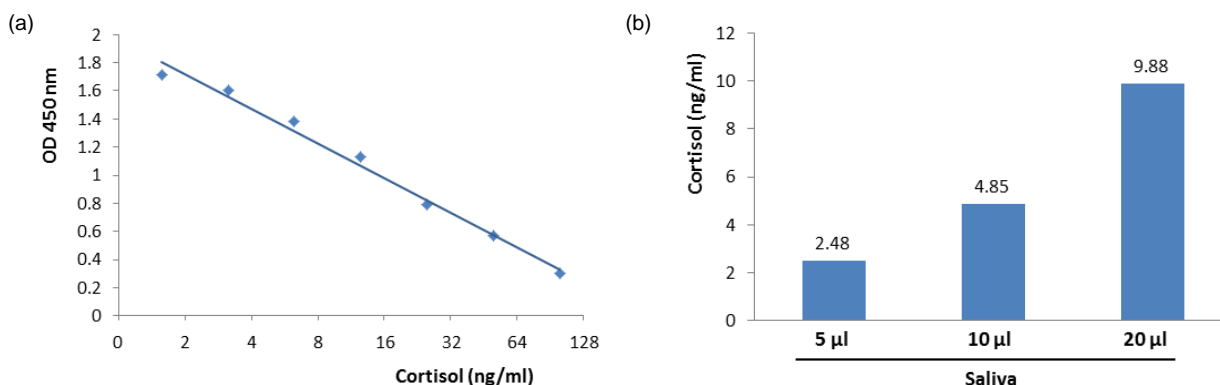
- Incubation time after addition of TMB substrate can be optimized to avoid over-development of color. Recommended absorbance for the 0 ng/ml cortisol standard is ~0.5-0.8 at 650 nm.
  - Optional: Prepare one parallel well for background control and add TMB Substrate.
- Add 100  $\mu$ l of Stop Solution into each well including background control and mix with gentle shaking. Remove air bubbles if any. Measure absorbance 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.

$$\text{Sample Cortisol Concentration} = B \times D = \text{ng/ml}$$

Where **B** is cortisol concentration in the sample well from Standard Curve.

**D** is sample dilution factor



**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 saliva (sample volume adjusted to 20  $\mu$ l for the assay) following the kit protocol.

**IX. RELATED PRODUCTS:**

- |   |   |
|---|---|
| Cortisol (human/mouse/rat) ELISA Kit (K7430)                  | Growth Hormone (human) ELISA Kit (K7412)              |
| Prolactin (human) ELISA Kit (K4687)                           | Luteinizing Hormone [LH] (human) ELISA Kit (K7426)    |
| Prolactin (mouse/rat) ELISA Kit (K4688)                       | Thyroid Stimulating Hormone (human) ELISA Kit (K7411) |
| Adrenocorticotrophic Hormone (ACTH) (human) ELISA Kit (K4003) |   |

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