Catalog #80957

V.2

Cortisol (Saliva) ELISA Kit Instructions

For the quantitative determination of
Cortisol in human saliva

Catalog #80957
96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Cortisol (Saliva) ELISA kit is for the quantitative determination of cortisol in human saliva. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Cortisol is a glucocorticoid hormone that is secreted in the adrenal cortex, and it has several roles in the body. It helps regulate metabolism by influencing gluconeogenesis and glycogenesis. It reduces inflammation by affecting the production of intereukins such as IL-1, IL-4, IL-10, and IL-12. It has roles in learning and memory by working with epinephrine. Additionally, it influences bone formation, gastric secretions, and mood. Given the wide-ranging implications of cortisol levels in the body, cortisol is being studied across several areas of research.

In the body, most circulating cortisol is bound to cortisol binding globulin or albumin and only about 1-2% is unbound or 'free.' This 'free' cortisol is considered to be the biologically active portion. In the absence of unusually large amounts of cortisol binding globulin, cortisol saliva correlates to a 'free' cortisol measurement and is an easy and unobtrusive method to collect samples.

C. Principle of the Assay

The Cortisol ELISA kit is a competitive ELISA for cortisol. It utilizes a specific antibody immobilized onto microplate wells and a labeled antigen conjugated with HRP. The unlabeled antigen present in the sample, standard, or control is incubated in the microplate well with HRP labeled antigen. Both the labeled and unlabeled antigen compete for binding to the immobilized antibody. After incubation, all unbound antigen is removed via a wash step. Subsequently, a substrate solution and stop solution are added, and cortisol levels of the samples can be measured by color intensity.

D. Kit Storage

1. Upon receipt of the Cortisol (Saliva) ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
STD1-6	Standards	6 vials
CON1-2	Controls	2 vials
HRP	Cortisol-HRP Conjugate Concentrate	1 x 300 μL
WASH	Wash Buffer (10X Concentrate)	1 x 50 mL
ASSAY	Assay Buffer	1 x 15 mL
SUB	Substrate Solution	1 x 16 mL
STOP	Stop Solution	1 x 6 mL

E.2. Materials required but not provided

Micropipettes and disposable tips
Deionized or distilled water
Microplate reader (capable of reading at 450 nm)
Orbital shaker
Centrifuge
Clean test tubes

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.
5. Do not let the substrate or stop solution come in contact with metal parts including aluminum foil.

G. Maximizing Kit Performance

1. Given the small sample volumes required (50 µL), pipetting should be done as carefully as possible. A high quality 100 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each standard, control, and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Collect saliva prior to eating, drinking, or brushing teeth. Rinse mouth with water before sample collection, and collect the samples in clean tubes. Saliva samples should be capped and stored at 2-8°C for up to 24 hours prior to assaying. For longer term storage, samples should be stored at -20°C. Avoid repeated freeze-thaw cycles of samples. Avoid blood contaminated samples. Samples containing azide or thimerosal are not compatible with this kit.

I. Assay Procedure

All reagents, unless otherwise noted, are stable until the expiration date at 2-8°C once opened.

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture.
2. Standards 1-6
   Standards are provided in liquid form with concentrations ranging from 0 ng/mL to 100 ng/mL. Standards, once opened, are stable for two weeks at 2-8°C. For longer term storage, opened standards should be frozen. Standards should be not be repeatedly thawed, so standards should be appropriately

aliquoted in appropriate volumes prior to being frozen. Standards are provided in the following approximate concentrations: 0, 1, 3, 10, 30, and 100 ng/mL, and exact values are listed on each bottle.

3. Controls 1-2
   Controls are provided in liquid form (0.6mL) with target value and ranges included on their labels. Controls, once opened, are stable for two weeks at 2-8°C. For longer term storage, controls should be frozen. Controls should not be repeatedly thawed, so controls should be aliquoted in appropriate volumes prior to being frozen.
4. Cortisol-HRP Conjugate Concentrate (50X Concentrated)
   The conjugate has to be diluted 1:50 in assay buffer prior to use. For example, 40 µL of HRP conjugate must be diluted in 2 mL of assay buffer. Dilute only as needed and discard any unused working conjugate.
5. Wash Buffer (10X Concentrated)
   The wash buffer has to be diluted 1:10 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 450 mL of distilled or deionized water. Dilute only as needed.
6. Assay Buffer
   Provided as ready to use.
7. Substrate Solution
   Provided as ready to use.
8. Stop Solution
   Provided as ready to use.

I.2. Assay procedure

Prior to running the assay, freeze the saliva samples. When ready to use, thaw samples and centrifuge. Collect the supernatants in clean tubes for use in the assay.

All reagents should be brought to room temperature for at least 30 minutes before use. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling. Remove the desired number of well strips from the foil pouch and store the remaining.

1. In the desired well, add 50 µL of sample, standard, or control.
2. In each well, add 100 µL of working Cortisol-HRP conjugate.
3. Incubate on a plate shaker (200 rpm) for 45 minutes at ambient temperature.
4. Aspirate well contents and wash three times using 300 µL of prepared Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
5. Add 150 µL of Substrate Solution in each well.
6. Incubate the plate for 15 minutes at ambient temperature in a dark room.
7. Stop the reaction by adding 50 µL of Stop Solution per well.
8. Measure the optical density within 20 minutes using a plate reader at 450 nm.

I.3. Determining the Cortisol concentration

1. Using computer software, construct the cortisol calibration curve by plotting the mean optical density for each standard on the Y axis versus the corresponding cortisol concentration on the X axis. A four or five parameter curve fit is suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*

2. Cortisol concentrations in the samples or controls are interpolated using the calibration curve and mean optical density for each sample. The cortisol concentration is expressed in ng/mL.
   Note: *Samples with a reading higher than 100 ng/mL should be diluted with the 0 ng/mL standard and rerun. Extrapolated values need to be multiplied by the dilution factor to calculate the cortisol concentration. Do not use a dilution factor higher than 1:8.*

J. Performance characteristics

J.1. Assay range

The Cortisol ELISA Kit has an assay range from 1 – 100 ng/mL.

J.2. Precision

The assay has an average within-run and total precision of CV $\leq$ 10%.

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