



Cortisol (Human) ELISA Kit

Catalog Number KA0918

96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

The Cortisol (Human) ELISA Kit is intended for the quantitative measurement of Cortisol in human serum or plasma.

Background

Cortisol (hydrocortisone, compound F) is the most potent glucocorticoid synthesized from cholesterol. Cortisol is found in the blood either as free Cortisol, or bound to corticosteroid-binding globulin (CBG). Cortisol production has an ACTH-dependent circadian rhythm with peak levels in the early morning and a nadir at night. The factors controlling this circadian rhythm are not completely defined. Serum levels are highest in the early morning and decrease throughout the day. In the metabolic aspect, Cortisol promotes gluconeogenesis, liver glycogen deposition, and the reduction of glucose utilization. Immunologically, Cortisol functions as an important anti inflammatory, and plays a role in hypersensitivity, immunosuppression, and disease resistance. It has also been shown that plasma Cortisol levels elevate in response to stress. Abnormal Cortisol levels are seen with a variety of different conditions: with adrenal tumors, prostate cancer, depression, and schizophrenia. Elevated Cortisol levels and lack of diurnal variation have been identified in patients with Cushing's disease.

Principle of the Assay

The Cortisol is a solid phase competitive ELISA. The samples and Cortisol enzyme conjugate are added to the wells coated with anti-Cortisol monoclonal antibody. Cortisol in the sample competes with a Cortisol enzyme conjugate for binding sites. Unbound Cortisol and Cortisol enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of Cortisol in the samples. A standard curve is prepared relating color intensity to the concentration of the Cortisol.

General Information

Materials Supplied

List of component

Component	96 Tests
Microwells coated with Cortisol MAb	12x8x1
Cortisol Standard: 7 vials (Ready to use)	0.5 ml
Enzyme Conjugate (20X)	1.2 ml
TMB Substrate: 1 bottle (Ready to use)	12 ml
Stop Solution: 1 bottle (Ready to use)	12 ml
20X Wash concentrate: 1 bottle	25 ml
Assay Diluent	24 ml

Storage Instruction

- ✓ Store the kit at 2-8 °C.
- ✓ Keep microwells sealed in a dry bag with desiccants.
- ✓ The reagents are stable until expiration of the kit.
- ✓ Do not expose test reagents to heat, sun, or strong light

Materials Required but Not Supplied

- ✓ Distilled or deionized water
- ✓ Precision pipettes
- ✓ Disposable pipette tips
- ✓ ELISA reader capable of reading absorbance at 450 nm
- ✓ Absorbance paper or paper towel
- ✓ Graph paper

Precautions for Use

- ✓ Precautions
 - Potential biohazardous materials:

The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in

Microbiological and Biomedical Laboratories." 1984.

- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
 - The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
 - It is recommended that standard, control and serum samples be run in duplicate.
 - Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
- ✓ Limitation of the test
- The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
 - Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

Assay Protocol

Reagent Preparation

- ✓ Cortisol-enzyme Conjugate Solution: Dilute the Cortisol enzyme conjugate 1:21 with assay diluent in a suitable container. For example, dilute 100 µl of conjugate with 2 ml of assay diluent buffer for 10 wells (A slight excess of solution is made).
- ✓ Wash Buffer: Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Sample Preparation

- ✓ Collect blood specimens and separate the serum immediately.
- ✓ Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
- ✓ Avoid multiple freeze-thaw cycles.
- ✓ Prior to assay, frozen sera should be completely thawed and mixed well.
- ✓ Do not use grossly lipemic specimens.

Assay Procedure

Prior to assay, allow reagents to stand room temperature.

Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Pipette 25 µl of Cortisol standards, control and patient's sera.
3. Add 200 µl of Cortisol Enzyme Conjugate to all wells.
4. Thoroughly mix for 10 seconds.
5. Incubate for 60 minutes at room temperature (18-26°C).
6. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
7. Add 100 µl of TMB substrate to all wells.
8. Incubate for 15 minutes at room temperature (18-26°C).
9. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 20 minutes after adding the stop solution.

Data Analysis

Calculation of Results

The standard curve is constructed as follows:

- ✓ Check Cortisol standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
- ✓ To construct the standard curve, plot the absorbance for Cortisol standards (vertical axis) versus Cortisol standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
- ✓ Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample

The following data represent a typical dose/response curve.

	OD 450 nm	Conc. ng/mL
Standard 1	2.36	0
Standard 2	1.76	20
Standard 3	1.10	50
Standard 4	0.65	100
Standard 5	0.29	200
Standard 6	0.13	400
Standard 7	0.08	800

Performance Characteristics

- ✓ Expected Values

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for Cortisol may be used as initial guideline ranges only:

Classification	ng/ml
8:00 AM-10:00 AM	50-230
4:00 PM	30-150

- ✓ Correlation with a Reference ELISA kit:

A total of 86 sera were tested by Cortisol ELISA and a reference ELISA kit. Results were as follow:

Correlation	Slope	Intercept
0.95	0.96	0.5

✓ Precision

- Intra-Assay Precision was determined by assaying 16 replicates of each of three sera; low, normal, and high.

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation %
1	16	40.1	2.54	6.3
2	16	59.4	5.57	9.4
3	16	165.5	10.23	6.2

- Inter-Assay Precision was determined by assaying duplicates of three serum pools in 10 separate runs, using a standard curve constructed for each run.

Serum	No. of Runs	Mean ng/ml	Standard Deviation	Coefficient of Variation %
1	10	52	7.8	15
2	10	87	8.9	10.2
3	10	156	13.5	8.6

✓ Sensitivity

The specificity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	1.38	0.06	1.5 ng/ml

✓ Specificity

Steroid	Crossreactivity
Cortisol	100%
Prednisolone	5.1%
11-Deoxycortisol	0.6%
Corticosterone	0.3%
11-Deoxycorticosterone	<0.1%
Progesterone	<0.1%
17-OH-Progesterone	<0.1%
Estradiol	<0.1%
Testosterone	<0.1%

Resource

Reference

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Plate Layout

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