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Cat#: CO368S (96 Tests)
For Order and Inquiries, please contact
Calbiotech Inc.,
1935 Cordell Ct. El Cajon, CA 92020
Tel (619) 660-6162, Fax (619) 660-6970,
www.calbiotech.com

CALBIOTECH
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Cortisol ELISA

Catalog No. CO368S (96 Tests)

INTENDED USE

The Calbiotech, Inc. (CBI) Cortisol ELISA Kit is intended for the quantitative measurement of Cortisol in human serum or plasma. For research use only. Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION

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 TEST

The Calbiotech, Inc. Cortisol test kit is a solid phase competitive ELISA. The samples, working Cortisol-HRP Conjugate and anti-cortisol-biotin solution are added to the wells coated with streptavidin. Cortisol in the patient's serum competes with the cortisol enzyme (HRP) 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.

MATERIALS PROVIDED		96 Tests
1.	Streptavidin coated microwells	12x8x1
2.	Cortisol Standard: 6 vials (ready to use)	0.5 ml
3.	Biotin Reagent: 1 bottle (ready to use)	7 ml
4.	Enzyme Conjugate (20X)	0.7 ml
5.	Assay Diluent: 1 bottle	12 ml
6.	TMB Substrate: 1 bottle (ready to use)	12 ml
7.	Stop Solution: 1 bottle (ready to use)	12 ml
8.	20X Wash concentrate: 1 bottle	25 ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

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

WARNINGS AND PRECAUTIONS

1. 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, 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.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate.
5. 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.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. 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.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

1. **Cortisol-enzyme Conjugate Solution**
   Dilute the Cortisol enzyme conjugate 1:21 with assay diluent in a suitable container. For example, dilute 100µl of conjugate with 2ml of assay diluent buffer for 10 wells (A slight excess of solution is made).
2. **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 (20-25°C).

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at 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 50 µl of Biotin reagent to all wells.
4. Add 100µl of Cortisol Enzyme Conjugate to all wells.
5. Thoroughly mix for 10 seconds.
6. Incubate for 60 minutes at room temperature (20-25°C).
7. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
8. Add 100 µl of TMB substrate to all wells.
9. Incubate for 15 minutes at room temperature (20-25°C).
10. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
11. Read absorbance on ELISA Reader at 450 nm within 20 minutes after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. 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.
2. 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.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Data

	OD 450 nm	Conc. ng/mL
Std 1	2.77	0
Std 2	1.42	20
Std 3	0.79	50
Std 4	0.43	100
Std 5	0.22	200
Std 6	0.12	500