CORTISOL
ENZYME IMMUNOASSAY (ELISA) TEST KIT

Enzyme Immunoassay for the Quantitative Determination of Cortisol in Human Serum

Intended Use

The Cortisol ELISA Kit is intended for the quantitative measurement of Total Cortisol in human serum or plasmaby a microplate enzyme immunoassay.

Instruction

Cortisol is a corticosteroid hormone or glucocorticoid produced by the adrenal cortex that is part of the adrenal gland (in the Zona fasciculata and the Zona reticularis of the adrenal cortex). It is usually referred to as the "stress hormone" as it is involved in response to stress.

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). 90% of the cortisol is bound to CBG, around 7% to Albumin and the rest is free.

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 antiinflammatory.

and plays a role in hypersensitivity, immunosuppression, and disease resistance. It has also been shown that plasma Cortisol levels elevate in response to stree. 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 diumal variation have been identified in patients with Cushing's disease.

Elevated cortisol levels and lack of diumal variation have been identified in patients with Cushing's disease (ACTH hyper secretion). Elevated circulating cortisol levels have also been identified in patients with adrenal tumors. Low cortisol levels are found in primary adrenal insufficiency (e.g. adrenal hypoplasia, congenital adrenal hyperplasia, addison's disease) and in ACTH deficiency. Due to the normal circadian variation of cortisol levels, distinguishing normal and abnormally low cortisol levels can be difficult. Therefore, various tests to evaluate the pituitary-adrenal (ACTH-cortisol) axis, including insulin-induced hypoglucemia, short and log term ACTH stimulation,

CRFstimulation and artificial blockage of cortisol synthesis with metronome have been preformed. Cortisol response characteristics for each of these procedures have beenreported.

Test principle

The Cortisol ELISA kit is based on 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 patient's simple 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 TMB 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 and components Materials provided with the test kits:

• Cortisol Microtiter Wells (monoclonal anti- Cortiosol antibody Coated microtiter plate).96 wells

• Cortisol Standard set (Cortisol standards, 6 doses), containing 0, 20, 50, 100, 200 and 500 ng/ml of Cortisol, in liquid form (ready to use)	0.5 ml
• Cortisol two levels of control	0.5 ml
• Cortisol Conjugate Reagent	12 ml
• TMB Substrate	12 ml
• Stop Solution	12 ml
• Wash Buffer Concentrate (50X)	15 ml

Materials required but not provided:

• Disposable pipette tips
• Distilled water
• Vortex mixer
• Absorbent paper or paper towel
• Microtiter plate reader
• Precision pipettes: 5µl-200µl, 200-1000µl
• Graph paper

Specimen collection and preparation

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Specimens should be capped and may be stored up to 48 hours at 2-8ºC, prior to assaying. Specimens held for a longer time can be frozen at -20□C. Thawed samples must be mixed prior to testing.

Storage of test kits and instrumentation

1. Unopened test kits should be stored at 2-8ºC upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Reagent preparation

• All reagents should be brought to room temperatura (18-22ºC) and mixed by gently inverting or swirling prior to use. Do NOT induce foaming.
• Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

Assay procedure

1. Secure the desired number of coated wells in the holder. Dispense 25 µl of standards, serum, and controls into the appropriate wells. Gently but thoroughly mix for 10 seconds.
2. Dispens 100µl of Conjugate Reagent into each well, Mix gently for 30 seconds. It is very important to have a complete mixing in this setup. And incubation at room temperature for 60 mins.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual wáter droplets.
4. Dispense 100 µl of TMB substrate into each well. Gently mix for 10 seconds. Incubate at room temperature (18-22ºC), in the dark, for 20 minutes.
5. Stop the reaction by adding 100µl of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
6. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

Important Note:

1. The wash procedure is critical. Insufficient washing

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will result in poor precision and falsely elevated absorbance readings.

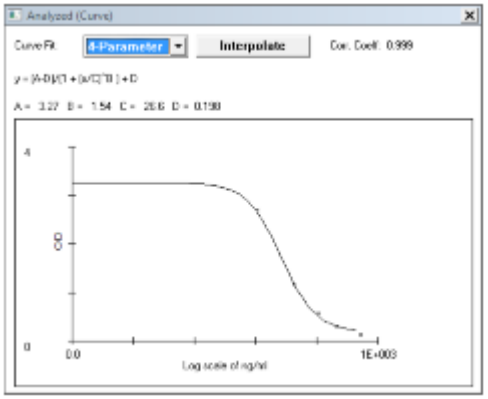
2. Duplication of all standards and specimens, although not required, is recommended.

3. It is recommended using the multiple channel pippets to avoide time effect. A full plate of 96 wells may be used if automated pipetting is available.

Calculation of results
Calculate the mean absorbance value for each set of Cortisol reference standards, specimens and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in units per ml on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of Cortisol in units per ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of standard curve
Results of a typical standard run with optical density reading at 450nm shown in the Y axis Cortisol concentrations shown in the X axis.

Cortisol Values (ng/ml)	Absorbance (450nm)
0.0	2.476
20.0	2.177
50.0	1.133
100.0	0.515
200.0	0.286
500.0	0.162



This standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her own standard curve and data.

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

Classification	ng/ml
8:00am - 10:00am	50~230
4:00pm	20~150

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend the range of expected values established by the Manufacturer only until an inhouse range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

Performance Characteristics

1) Precision Three concentration levels of Cortisol samples were used to determine the intra precision of Cortisol ELISA assay kit. The results were shown in the table below.

Concentrations	NO.	Mean (ng/ml)	S.D.	CV
Level 1	16	19.8	1.46	7.3 %
Level 2	16	67.3	4.43	6.6 %
Level 3	16	240.6	13.64	5.7 %

Three concentration levels of Cortisol samples were used to determine the inter precision of Cortisol ELISA assay kit.

Concentrations	N	Mean	S.D.	CV
Level 1	16	20.5	1.89	9.2%
Level 2	16	67.7	4.45	6.6%
Level 3	16	246.8	16.76	6.8%

2) Limit of Blank
The Limit of Blank (LOB) was calculated by measuring the blank several times and calculating 95th percentile of the distribution of the test values on truly blank samples deviate significantly from blank measurements. The LOB of this assay is estimated to be 2.23 ng/ml.

3) Limit of Detection
The minimum detectable concentration is 0.073 ng/ml. The sensitivity is definied as the mean plus two standard deviations of 20 replicated of the zero calibrator. The sensitivity is 3.71 ng/ml.

4) **Detection Range**
Limit of Quantitation (LOQ) is the lowest concentration at which the analyte cannot only be reliably detected but at which some predefined goal of bias and imprecision are met. The Detection Range is from 3.99 ng/ml to 500 ng/ml without need of sample
dilution.

Limitations
1) As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical
and laboratory findings have been evaluated.

2) Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies
for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.

3) This kit is designed for professional use only.

References
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PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET046