CE

DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302
Tel: (818) 591-3030 Fax: (818) 591-8383
onestep@rapidtest.com
technicalsupport@rapidtest.com
www.rapidtest.com

IVD	See external label	2°C-8°C	Σ=96 tests	REF	Cat # 6101Z

Cortisol ELISA

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Test	Cortisol ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Indirect ELISA: Antigen Coated Plate
Detection Range	0-60 µg/dL
Sample	10 µl serum
Specificity	100%
Sensitivity	0.36 µg/dL
Total Time	~90 min
Shelf Life	12 months

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

NAME AND INTENDED USE

The DAI CORTISOL Quantitative is a solid phase enzyme linked immunosorbent assay (ELISA). This test provides quantitative measurement of Cortisol in the human serum.
(For professional use only)

SUMMARY AND EXPLANATION OF TEST

Cortisol is the major glucocorticoid produced and secreted by the adrenal cortex. It affects the metabolism of protein, fat and carbohydrates; the maintenance of muscle and myocardial integrity; and the suppression of inflammatory and allergic activities. Production of cortisol from the adrenal cortex is dependent upon corticotrophin (ACTH), which is secreted by the anterior pituitary. The corticotrophin-releasing factor (CRF) that regulates ACTH is secreted by the hypothalamus and is responsive to Cortisol levels. Physical, psychological and surgical stress and diuranal variation will affect the rate of cortisol production1. Cortiscosteroid-binding globulin and albumin bind approximately 90% of the Cortisol secreted by the adrenal cortex. Bound Cortisol circulates in an available but temporarily inactive state. The physiological activity of cortisol depends upon levels of the small fraction of circulating unbound cortisol2. The measurement of cortisol levels aids in the diagnosis of normal and abnormal states of adrenal gland function. It is also helpful in the diagnosis of Cushing's disease (high cortisol) and Addison's diseases (low cortisol) 3, 4. The ACTH stimulation test is used to distinguish between primary and secondary adrenal insufficiency5. Suppression tests using dexamethasone and metyrapone are used to check the integrity of feedback system and are useful in the diagnosis of Cushing's disease6, 7.

PRINCIPLE OF THE ASSAY

The DAI CORTISOL Quantitative test is based on the principle of competitive solid phase enzyme immunoassay. The wells are coated with anti-Cortisol IgG. The samples, controls or standards are incubated simultaneously with Cortisol conjugated to enzyme horseradish peroxidase. After incubation, the samples, controls or standards competes with enzyme-labeled Cortisol to bind anti-Cortisol antibody in the wells. Unbound materials are removed by washing step. Upon addition of chromogen substrate, the color developed proportional to the amount of enzyme activity that in turn is inversely proportional to the amount of Cortisol in the sample. The intensity of the color is measured by using 450 nm microtitier plate readers. The concentrations of samples are obtained by reference to the standards.

WARNING AND PRECAUTION

1. The DAI CORTISOL Quantitative is designed for in vitro use only.
2. The components in this kit are intended for use as an integral unit.
3. The components of different lots should not be mixed. It is advised not to exchange strips of different plates even if of the same lot. The kit may have been shipped or stored under different\ conditions, and the characteristics of the plates may result slightly different.
4. Warning potential bio-hazardous material: Some reagents such standards contain human serum. The human serum used has been found negative for HIV and HCV antibodies as wells as for Hepatitis B surface antigen when tested with FDA licensed reagents. However, as there is no test method that can offer completeassurance that HIV, HCV and Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Bio-safety Level 2, as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.

STORAGE AND STABILITY

Store the kits at 2-8oC in the refrigerator. Keep microwells in dry bag with desiccants.

The reagents are stable until expiration of the kit. The calibrators and conjugate are stable for 8 weeks after first opening. Chromogen substrate solution should be colorless; if the solution turns blue, it must be replaced. Do not expose these reagents to strong light during storage or usage.

MATERIALS PROVIDED

1. Microwell strips (96 wells): anti-cortisol IgG coated wells.
2. Enzyme conjugate (11 mL): Cortisol conjugated to horseradish peroxidase.
3. Reference Standard Set (0.5 ml each vial): Prepared 0, 1, 3, 10, 30 and 60 µg/dL in the human serum base.
4. Low Control (0.5 mL) value ranges indicated on the vial.
5. High Control (0.5 mL) value range indicated on the vial
6. TMB Solution (11 mL): Buffer solution containing hydrogen peroxide and TMB
7. Washing Concentrate (10 mL) (100X): to prepare the working washing buffer by adding 10 ml concentrate in to 990 mL of distilled water.
8. Stop Solution: 2N HCL
9. Well holder for securing individual wells.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microwell reader with 450 nm.
2. Pipetor with disposable tips for 10 uL, and 100 uL.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting,and separating the serum by centrifugation at room temperature. If sera cannot be assayed immediately, they can be stored at 2 - 8oC or frozen for few days. Do not use hyperlipemic, hemolyzed, contaminated or heat inactivated sample as they may cause erroneous results.

PREPARATION FOR ASSAY

1. Before beginning the test, bring all specimens or reagents to room temperature and mix well.
2. Have all reagents and samples ready and organized before the starting the steps of the assay. Once the test is begun it must be performed without any interruption.
3. Use new disposable tips for each standard, control and specimen.

PROCEDURAL NOTE

1. Azide and thimerosal at concentration higher than 0.01% interfere in this test; therefore, the assay of control sera or samples containing the above compound may give high results.
2. It is very important to wash the microwell thoroughly and remove the last droplets of water to achieve the best results.
3. Pippet all reagents and samples into the bottom of the wells. Vortex-mixing or shaking of wells after sample and reagent pipetting is not required.
4. Absorbance is a function of the time and temperature of incubations. It is recommended to have all reagents and samples caps remove, all needed wells secured in holder and assigned. It will ensure the equal elapsed time for each pipetting without interruption.
5. For the same reason the size of the assay run should be limited. It is suggested running no more than 20 patient samples with a set of reference standards in duplicate.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.

2. Dispense 10 uL of standards, controls or serum samples into appropriate wells. Save one well for the blank (do not add any standards or enzyme conjugate).
3. Dispense 100 uL of cortisol-enzyme conjugate.
3. Incubate for 60 minutes at room temperature.
4. Remove incubation mixture and rinse the wells with wash buffer 5 times.
5. Dispense 100 uL of TMB Solution into each well including the blank well.
6. Incubate for 30 minutes at room temperature.
7. Stop reaction by adding 50 uL of 2 N HCl to each well and read O.D. at 450 nm with microwell reader.
8. Record absorbencies.

CALCULATION FO RESULTS

1. Calculate the index A/Ao x 100 for standards, controls and patient samples. A is the absorbance of each standard, control or patient sample and Ao is the average absorbance of the replicates of 0 ug/dL Cortisol standard.
2. Plot the concentration (X) of each reference standard against it's A/Ao x 100 index (Y) on the logit-log paper. Draw a point to point line through the mean of the duplicate point.
3. Obtain the value of patient Cortisol by standard curve. The following data is for demonstration purpose only and must not be used in place of data for each assay.

Calibrator Controls (μg/dL)	Absorbance	A/Aox100	Calculated Results (μg/dL)
0	2.549 2.452	100%	
1.0	2.173 2.051	84.5%	
3.0	1.479 1.380	57.2%	
10.0	0.694 0.732	28.5%	
30.0	0.443 0.399	16.8%	
60.0	0.317 0.246	11.3%	
Control 1	1.615 1.669	65.7%	2.2
Control 2	0.385 0.404	15.8%	32.1

LIMITATION

1. For diagnostic purpose, the Cortisol values should be used as an adjunct to other data available to the physician.
2. Due to high cross-reactivity of the antibody with prednisolone, this test is not suitable for the samples of patients who are being treated with prednisolone or prednison.
3. Crossly hemolyzed or lipemic samples may give erroneous results.
4. Sample Cortisol level above 60 ug/dL should be pre-diluted and re-tested.

EXPECTED VALUE

1. It is recommended that each laboratory should determine its own normal and abnormal range.
2. The following values can be used as preliminary guidelines until the laboratory establishes its own normal ranges.

Time	N	Range
AM	40	7-24
PM	27	5-13

3. Because of diurnal variation in normal substrates, serum or plasma, Cortisol levels are highest in the morning and lowest in the evening.
4. Serum cortisol levels after ACTH stimulation tests normally increase 2-3 times the basal value[8]. Dexamethasone or metyrapone suppression tests normally lower the basal value to 75-90%[9].
5. Assay values for plasma samples with heparin or EDTA may approximately 5- 10% lower than for serum.

PERFORMANCE CHARACTERISTICS

A. PRECISION

Intra-assay and Inter-assay coefficient of variation were evaluated at three different pooled serum controls.

Intra-Assay	Pool A	Pool B	Pool C
N	10	10	10
Mean	2.51	19.22	33.02
C.V. (%)	9.5%	6.1%	6.2%

Inter-Assay	Pool A	Pool B	Pool C
N	10	10	10
Mean	2.72	20.11	32.35
C.V. (%)	10.1%	8.7%	7.2%

B. ACCURACY

Recovery studies were performed by mixing an aliquot of pooled serum and cortisol standards. The cortisol values were measured and percentage of recovery was determined.

Initial Values µg/dL 200 µL	Concentration Spiked µg/dL 100 µL	Expected Values µg/dL	Observed Values µg/dL	Recovery
3.4	3.0	3.2	3.2	100
3.4	10.0	6.7	5.8	86
3.4	30.0	16.7	13.8	83
25.6	3.0	14.3	15.0	105
25.6	10.0	17.8	19.9	111
25.6	30.0	27.8	24.0	86

Correlation: The DAI Cortisol was compared with the commercial RIA kit. Linear regression analysis are as following:
N = 46
X = Commercial RIA kit
Y = 1.10X -1.43
R (correlation coefficient) = 0.96

C. Sensitivity

The minimal detectable concentration of Cortisol is estimated to be 0.36 µg/dL. The minimal detectable concentration is defined as the concentration of the Cortisol that corresponds to the absorbance that is

two standard deviation smaller than the mean absorbance value of twenty replicates determination of the zero concentration.

D. Specificity

Compounds	Percent Cross Reactivity	Compounds	Percent Cross Reactivity
Cortisol	100	Epiandrosterone	0.0
Cortisosterone	9.3	Estradiol	0.0
Cortisone	2.2	17 hydroxyprogesterone	1.0
Dehydroepiandrosterone	0.0	Prednisolene	33.3
11- Deoxycorticosterone	0.6	Prednisone	1.4
11-Deoxycortisol	3.8	Progesterone	0.1
Dexamethasone	0.3	Testosterone	0.1

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Tel: (818) 591-3030 Fax: (818) 591-8383

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