Species crossreactivity

ADI's human cortisol ELISA kit has not been validated by ADI for animals or other species. However, the kit may be optimized for species (mouse, rat, monkey etc) where the cortisol are within the detectable range of the kit.

Samples requirements

This kit is optimized for human serum samples. Other biological fluids such as culture medium, plasma, CSF can be tested as well.

Related Steroid Hormone ELISA kits available from ADI (Details and complete listing is posted at the web site)

ItemName	Cat #
Human Cortisol ELISA Kit	1850
Human Progesterone ELISA Kit	1860
Human Pregnolone	1865
Human Progesterone (saliva) ELISA	1870
Human Aldosterone ELISA Kit	1875
Human Testosterone ELISA Kit	1880
Human free Testosterone ELISA Kit	1885
Human Androstenedione ELISA Kit	1910
Human Androstenedione (saliva) ELISA	1915
Human Estradiol ELISA Kit	1920
Human Estrone ELISA Kit	1925
Human Dihydrotestosterone (DHT) ELISA Kit	1940
Human DHEA-sulphate (DHEA-S) ELISA Kit	1950

KIT PROFILE

Date received: **Cat #** 1850 **Lot #** _____ **Exp.** _____

Date kit opened _____ **Technician:** _____

Date used: _____ **# Strips used** _____ **# Remaining** _____

Date used: _____ **# Strips used** _____ **# Remaining** _____

Remarks _____

_____

Instruction Manual No. M-1850

CORTISOL

ELISA KIT Cat. No. 1850

For Quantitative Determination of Cortisol In Human or Animal Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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LIFE TECH Life Technologies™ Your Molecular & Cell Technology Partner

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

CORTISOL ELISA KIT # 1850 - Kit Contents: (reagents for 96 tests)

Components	
Anti-cortisol coated microwell strip plate (96 wells), #1851	1 Plate
Cortisol **Std. A** (0 ug/dl), 10 ml, #1852A	1 vial
Cortisol **Std. B** (0.5 ug/dl), 0.3 ml, #1852B	1 vial
Cortisol **Std. C** (2 ug/dl), 0.3 ml, #1852C	1 vial
Cortisol **Std. D** (5 ug/dl), 0.3 ml, #1852D	1 vial
Cortisol **Std. E** (10 ug/dl), 0.3 ml, #1852E	1 vial
Cortisol **Std. F** (30 ug/dl), 0.3 ml, #1852F	1 vial
Cortisol **Std. G** (60 ug/dl), 0.3 ml, #1852G All standards are provided in human serum base	1 vial
Cortisol **Low Control Serum**, 0.3 ml (#1852-LC) 1 vial	
Cortisol **High Control Serum**, 0.3 ml (#1852-HC) 1 vial	
Cortisol-HRP **Conjugate (100X)**; 0.3 ml ,#1853	1 vial
Assay Buffer, 15 ml. #1854	1 bottle
Wash buffer (10X), 50 ml, WB-10 Dilute 1:10 with distilled water,	1 bottle
HRP **substrate Soln**; 16 ml, TMB-1850	1 bottle
Stop solution, 6 ml, ST-10	1 bottle
Complete Instruction Manual	M1850

Introduction

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), which is regulated by the hypothalamus and is responsive to Cortisol levels. Physical psychological and surgical stress and diurnal variations will affect the rate of Cortisol production.

Corticosteroid-binding globulin (CBG) 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 the small fraction of circulating unbound Cortisol.

The measurement of cortisol levels aids in the diagnosis of normal and abnormal states of adrenal gland functions. It is also helpful in the diagnosis of Cushing's disease (high cortisol) and Addison's disease (low Cortisol). The ACTH stimulation test is used to distinguish between primary and secondary adrenal insufficiency. Suppression tests using dexamethasone and metyrapone are used to check the integrity of feedback system and are useful in the diagnosis of Cushing's disease.

ADI's Cortisol ELISA kit provides for the measurement of Cortisol in serum of human or animal.

2. PRECISION

Intra-assay precision:

	Pool A	Pool B	Pool C
Mean (ug/dl)	1.44	14.06	37.55
S.D.(ug/dl)	0.14	0.41	1.87
C.V. (%)	9.4	2.9	5.0

Inter-assay precision:

	Pool A	Pool B	Pool C
Mean (ug/ml)	1.60	15.01	38.18
S.D.(ug/dl)	0.13	0.74	1.43
C.V. (%)	8.1	5.0	3.8

3. ACCURACY

Mixing an aliquot of pooled serum and Cortisol standard performed recovery studies. The Cortisol values were measured and % of recovery was determined.

Initial Values (ug/dl) (200 ul)	Conc. spiked (ug/dl) (100 ul)	Expected values (ug/dl)	Observed values (ug/dl)	Recovery (%)
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
3.4	10.0	6.7	5.8	86
28.4	30.0	29.2	28.0	96

4. Linearity of Dilution

Three samples (10.94, 18.92, and 42 ug/dl) were diluted 1:2, 1:4, 1:8 with calibrator A and tested for cortisol conc. Recoveries with 90-116%.

5. Specificity

The following compounds were tested for crossreactivity of the assay: Cortisol (100%), Corticosterone (9.3%), Cortisone (2.2%) 11-Deoxycorticosterone (0.6%), 11-deoxycortisol-a-hydroxyCortisol (3.6%), Dexamethasone (0.3%), Epiandrosterone (0.0%), 17-a-hydroxyprogesterone (1.0%), Prednisolone (13.6%), Prednisone (1.4%), Progesterone, Testosterone, Estradiol (<0.1%).

6. Sensitivity

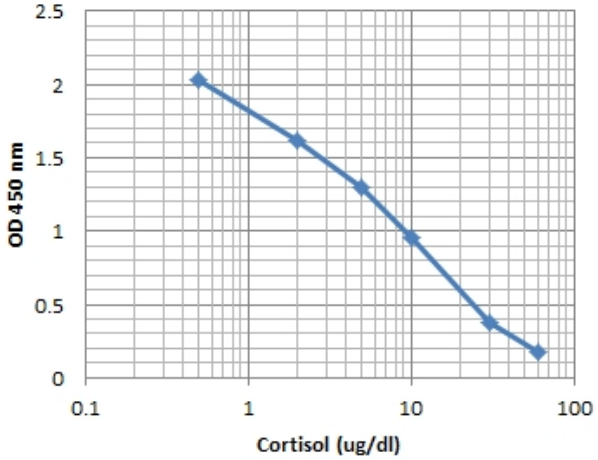
The minimal detectable concn. of cortisol is estimated to be **0.4 ug/dl**. The minimal detectable concn. is defines as the concn. of cortisol which corresponds to the absorbance that is 2 S.D. smaller than the mean abs. Value of the zero std.

General References: Travis JC (1976) Plasma cortisol Rx: RIA for Physicians V1, No 8; Vecsie P (1974) In Methods of Hormone RIA AP, NY, 394; Greig W (1966) J Endocrinol. 34, 411; Spark R (1971) Ann. Int. Med. 75, 717.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Net Mean $A_{450\ nm}$
A1, A2	**Std. A** (0 ug/dl)	2.223
B1, B2	**Std. B** (0.5 ug/dl)	2.036
C1, C2	**Std. C** (2 ug/dl)	1.178
D1, D2	**Std. D** (5 ug/dl)	1.395
E1, E2	**Std. E** (10 ug/dl)	0.959
F1, F2	**Std. F** (30 ug/dl)	0.379
G1, G2	**Std. G** (60 ug/dl)	0.175

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values.



*Kit-spec-XL
A typical std. assay curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

1. Calculate the net mean OD from the duplicates of standards, controls, and patients samples.
2. Draw the calibrator curve on semi-log pape with the mean A450 values on the Y-axis and the concn on X-axis as shown above. A is the absorbance of each std. Draw a point-to-point line through the mean of the duplicate point. If ELISA reader software is being used, we recommend 4-paramter or 5-parameter curve.
3. Obtain the value of sample Cortisol by standard curve.
4. If samples read more than 60 ug/dl then diluted with the calibrator A at a dilution no more than 1:18 and multiply the results with the dilution factor.

PRINCIPLE OF THE TEST

Cortisol ELISA kit is based upon competitive solid phase ELISA. The patient sample competes with enzyme-linked Cortisol for a fixed and limited number of antibody binding sites on the coated plates. In the assay, the Cortisol standard or samples sera are incubated with Cortisol-HRP conjugate in the anti-cortisol coated wells. In this solid-phase system, the antibody bound Cortisol will remain on the well while unbound Cortisol will be removed by washing. A color (blue) is developed when the substrate, TMB is mixed with the antibody bound Cortisol-HRP conjugate. After a short incubation, the enzyme reaction is stopped (blue color turns yellow) and the intensity of the color (yellow) is measured using an ELISA plate reader. The color is inversely proportional to the concentration of Cortisol in the sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (10-200 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Cortisol ELISA test is intended for *in vitro research* use only. The reagents contain proclin-300 (0.1% v/v) as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses; therefore, sera should be handled with appropriate precautions.

MSDS

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, these could be stored at -20^{O}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. Sodium azide and merthiolate at concentration >0.01% interfere in this test and may give higher results.

Preparation of reagents:

Dilute HRP-conjugate stock in assay buffer **1:100** eg; **20 ul of HRP in 2 ml** of assay buffer or **120 ul of HRP stock in 12 ml of assay buffer** for the whole plate. Prepare in required amounts only. Do not store diluted working stock and return the stock conjugate at 4oC.

Wash buffer is supplied as 10x stock. **Dilute 50 ml stock into 450 ml de-ionized or distilled water,** mix, and store at room temp for 1-2 weeks. It can be stored at 4oC for long term storage.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. HRP substrate buffer (solution A) and HRP substrate (solution B) should be colorless at the time of use. If solutions have turned light blue in color, these should be replaced. Do not expose these solutions to strong light during storage or use. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE - *ALLOW **ALL** REAGENTS TO REACH ROOM TEMPERATURE (25-30oC) BEFORE USE. Addition of cold reagents will reduce reaction rate and less color.* **Dilute wash buffer (1:10) with distilled water (50 ml stock in 450 ml).** Store at 4oC until use. **Dilute HRP-conjugate 1:100** in assay buffer eg; **20 ul of HRP in 2 ml** of assay buffer.

1. Remove required # strips and arrange them on the ELISA frame. Any used strips can be stored in the supplied plastic bag with the desiccant at 4oC. The ELISA plate frame can be saved after the test to be used again if partial plate was used for the assay.

2. Pipet **20 ul** of standards, control, and serum samples into appropriate wells in *duplicate*. Immediately add **100 ul** of **Cortisol-HRP conjugate** into each well. Mix gently for 5-10 seconds. Cover the plate and **incubate on a plate shaker (approx. 200 rpm) for 45 min at room temperature**.

3. Remove reaction mixture and **wash 3-times with 1X wash buffer** (300 ul/well/wash). We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Dispense **150 ul TMB substrate per well.** Mix gently. Blue color develops in standards and samples. Cover the plate and incubate on a plate shaker for **15-20 minutes** at room temperature. It is possible to vary the incubation time by 5 $\pm$ min to attain maximum color of A450=2.0-2.5.

5. Stop the reaction by adding **50 ul of stopping** solution to all wells**.** Mix gently. Blue color turns yellow. Measure the absorbance at 450 nm using an ELISA reader within 30 min.

NOTES - Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. Keep the incubation time for each well the same by adding the reagents in identical sequence. Do not touch the bottom of the wells.

DILUTION OF SAMPLES and LIMITATIONS

It is recommended that each laboratory must determine its own normal and abnormal ranges. Extrapolation of Cortisol values beyond the standard curve may yield variable results. Samples containing >60 ug/dl Cortisol can be diluted with 0 standard and retested. Calibrators and controls from other manufacturers may contain serum preservatives incompatible with ADI's ELISA reagents should not be used. Whenever laboratory data conflict with clinical findings or impressions, clinical judgment should be exercised and additional evaluation undertaken.

Due to high crossreactivity of the antibody with prednisolone, this test is not suitable for the samples of patients who are being treated with prednisolone or prednison. Grossly hemolyzed or lipemic samples may give erroneous results.

INTERPRETATION EXPECTED VALUE

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

WOMEN	Mean	Normal Range
A.M.	15.59	3.95-27.23 ug/dl
P.M.	5.93	1.45-10.41 ug/dl

2. Because of diurnal variations in normal substrates, serum, or plasma cortisol levels are highest in the morning and lowest in the evening.

3. Serum cortisol levels after ACTH stimulation tests normally increase 2-3 times the basal value. Dexamethasone or metyrapone suppression tests normally lower eh basal value to 75-90%.

4. Assay Values for plasma samples with heparin or EDTA may be approximately 5-10% lower than for serum.

Animal Species Testing

Steroid hormones are the same in all species. This kit has been used in animal (mouse) samples.

Bhat BG, 2007, Antisense inhibition of 11βhydroxysteroid dehydrogenase type 1 improves diabetes in a novel cortisone-induced diabetic KK mouse model, Biochemical and Biophysical Research Communications, 365, 740-745

We recommend using the same protocol for animal cortisol and then making further adjustments in terms of sample volume.