



Cortisol ELISA assay

Catalog: IB69100

INTENDED USE

This ELISA assay provides quantitative measurement of Cortisol in human serum. (For *in-vitro* diagnostic 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, and psychological stress as well as diurnal variation will affect the rate of cortisol production¹.

Corticosteroid-binding globulin and albumin bind approximately 90% of the cortisol secreted by the adrenal cortex. Bound cortisol circulates in an inactive state. The physiological activity of cortisol depends upon levels of the small fraction of circulating unbound cortisol².

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 (elevated cortisol) and Addison's diseases (depressed 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 the feedback system and are useful in the diagnosis of Cushing's disease^{6,7}.

PRINCIPLE OF THE ASSAY

The IBL-America serum cortisol assay is based on the competitive binding principal and is a solid phase enzyme immunoassay. The wells are coated with anti-cortisol IgG. The samples, controls or standards are incubated simultaneously with cortisol conjugated to the enzyme horseradish peroxidase. During incubation, the samples, controls or standards compete with enzyme-labeled cortisol to bind anti-cortisol antibody immobilized on the wells. Unbound materials are removed by washing. Upon addition of chromogen substrate, color development will occur 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 using a spectrophotometer equipped with a 450 nm filter. The concentrations of samples are obtained by reference to the standards.

WARNINGS AND PRECAUTIONS

1. This assay is designed for *in-vitro* diagnostic use only.
2. The components in this kit

3. are intended for use as an integral unit without substitution.
3. Do not interchange kit components from different lot numbers.
4. Warning potential bio-hazardous material: *Some reagents such as standards contain human serum. Any 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 complete assurance that HIV, HCV and Hepatitis B virus or other infectious agents are absent, the kit 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 kits refrigerated at 2-8°C. Always store microwells in a sealed bag with desiccant. 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 (12 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 human serum.
4. Low Control (0.5mL) see vial label for range.
5. High Control (0.5mL) see vial label for range.
6. Substrate Solution (Tetramethylbenzidine) (11 mL).
7. Wash Buffer Concentrate (10 mL)(100X): Requires dilution prior to use.
8. Stop Solution: 2 N HCl.
9. Microplate frame for securing individual wells.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water.
2. Microplate spectrophotometer with 450nm filter.
3. Automated microplate washer or multi-channel micro-pipet.
4. Pipettors with disposable tips for 10 µL, and 100 µL volumes

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If sera cannot be assayed immediately, they can be stored at 2 - 8°C for 2-4 days or frozen for up to six months. Do not use lipemic, hemolyzed, contaminated or heat inactivated samples as they may cause erroneous results.

PREPARATION FOR ASSAY

1. Prepare working dilution of wash buffer by adding 10 mL of concentrate to 990mL distilled or deionized water. CAUTION: If wash buffer concentrate has crystallized, it must be warmed gently to re-dissolve prior to dilution.

Diluted wash buffer is stable at 2-8°C for 30 days.

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

PROCEDURAL NOTES

- Sodium azide and thimerosal at concentrations higher than 0.01% interfere with this assay. Testing control sera or samples containing high levels of these compounds is not recommended.
- It is very important to wash the microwells thoroughly and remove residual moisture by inverting the microplate and tapping it sharply onto absorbent material after the final wash repetition.
- Pipet all reagents and samples into the bottom of the microwells. Vortex-mixing or shaking of wells after sample and reagent pipetting is not required.
- Absorbance is a function of the time and temperature of incubations. It is recommended that all reagent and sample vial caps be removed and all microwells organized in the microplate frame prior to pipetting to minimize elapsed time for each pipetting step.
- Noticeable assay drift can be an issue with any ELISA assay if excessive time elapses during the initial loading of standards, controls and samples into the plate. Care should

always be taken to load the plate as quickly/efficiently as possible and without interruption.

- All standards, controls and unknown samples should be run in duplicate.

ASSAY PROCEDURE

- Secure the desired number of coated microwells in the plate frame. Provide a well for the assay blank.
- Dispense 10 uL of standards, controls or serum samples into appropriate duplicate wells. Save one well for the blank (do not add sample or enzyme conjugate).
- Dispense 100 uL of HRP-conjugate into every well except the blank.
- Incubate for 60 minutes at room temperature.
- Remove incubation mixture and dispense 300ul of diluted wash buffer into every well, including the blank. Remove the wash buffer and repeat for a total of five repetitions. After removal of the final wash buffer, invert the plate and tap sharply onto absorbent material to remove any residual moisture from the wells.
- Dispense 100 uL of TMB substrate into each well including the blank well.
- Incubate covered or protected from light for 30 minutes at room temperature.
- Add 50 uL of Stop Solution to each well and read at 450 nm with spectrophotometer within 10 minutes.

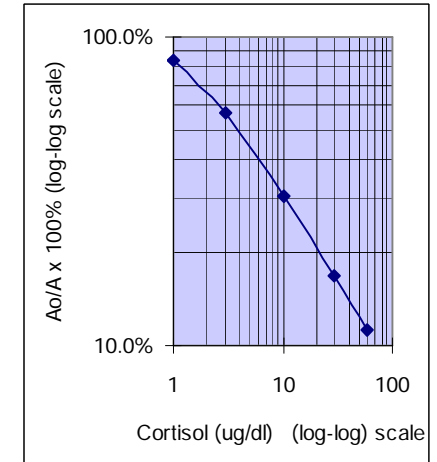
CALCULATION OF RESULTS

- Assay blank values to be subtracted from all other absorbance values.
- Calculate the index $A/A_o \times 100$ for standards, controls

and patient samples. A is the absorbance of each standard, control or patient sample and A_o is the average absorbance of the replicates of 0 ug/dL cortisol standard.

- Plot the concentration (X) of each reference standard against its $A/A_o \times 100$ index (Y) on the logit-log paper. Draw a point to point line through the mean of the duplicate point.
- 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 (ug/dL)	Absrb (450 nm)	A/Ao x 100%	Calculated Results (ug/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



LIMITATIONS

- Cortisol values derived from this assay should be used only in conjunction with other diagnostics available to the physician.
- Due to high cross-reactivity of the antibody with prednisolone, this test is not suitable for use with samples of patients who are being treated with prednisolone or prednisone.
- Grossly hemolyzed or lipemic samples may give erroneous results.
- Sample cortisol levels above 60 ug/dL should be pre-diluted and re-tested.

EXPECTED VALUES

- It is recommended that each laboratory establish their own normal and abnormal ranges.
- The following values are suggested only as preliminary guidelines until the laboratory establishes its own normal ranges.

Time	N	Normal Range (ug/dL)
A.M.	40	7 - 24
P.M.	27	5 - 13

- Because of typical diurnal variation, serum cortisol levels are highest in the morning and lowest in the evening.
- Serum cortisol levels after ACTH stimulation tests normally increase 2-3 times the basal value⁸. Dexamethasone or metyrapone suppression tests normally lower observed cortisol values to 75-90% of the basal value⁹.
- Assay values for plasma samples with heparin or EDTA may be approximately 5-10% lower than for serum.

PERFORMANCE CHARACTERISTICS

A. PRECISION

Intra-assay and Inter-assay coefficients of variation were evaluated with three different pooled serum controls.

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

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

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 ug/dL	Conc. Spiked ug/dL	Expctd Values ug/dL	Obsrvd Values ug/dL	Recvry (%)
200 uL	100 uL			
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 IBL-America cortisol ELISA assay was compared with a commercially available RIA kit. Linear regression analysis is:

N = 46
 X = Commercial RIA kit
 Y = IBL-America serum cortisol ELISA assay
 $Y = 1.10X - 1.43$
 R (correlation coefficient) = 0.96

C. SENSITIVITY

The minimum detectable concentration of cortisol is determined to be 0.36 ug/dL. The minimum detectable concentration is defined as that concentration of cortisol corresponding to the absorbance that is two standard deviations below the mean absorbance value of twenty replicate determinations of the zero standard.

D. SPECIFICITY

The following compounds were tested for cross reactivity:

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

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IBL-America, Inc.
 8201 Central Avenue, NE, Suite P
 Minneapolis, MN 55432
 Tel: (763)780-2955
 FAX: (763)-780-2988
 Website: www.IBL-America.com