EAGLE BIOSCIENCES

Cortisol ELISA Assay Kit

Catalog Number:
COR31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 2.1 (29 APR 24)

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INTENDED USE

The Eagle Biosciences Cortisol ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of Cortisol in human serum by an enzyme immunoassay. The Eagle Biosciences Cortisol ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

Cortisol is the most abundant circulating steroid and the major glucocorticoid secreted by the adrenal cortex. Cortisol is physiologically effective in blood pressure maintenance and anti-inflammatory activity. It is also involved in calcium absorption, gluconeogenesis as well as the secretion of gastric acid and pepsin. It is increased under stress situations, physical exercise and external administration of ACTH. Measurement of cortisol levels in general can be used as an indicator of adrenal function and the differential diagnosis of Addison's and Cushing's diseases as well as adrenal hyperplasia and carcinoma.

Most circulating cortisol is bound to cortisol binding globulin or transcortin and albumin. The free cortisol, which is considered the active part of blood, is about 1–2%. In the absence of appreciable amounts of the cortisol binding proteins in saliva, salivary cortisol is considered to be free and shows a diurnal rhythm with the highest levels in the morning and the lowest levels at night.

PRINCIPLE OF THE ASSAY

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed is inversely proportional to the concentration of cortisol in the sample. A set of standards is used to plot a standard curve from which the amount of cortisol in patient samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the wells will affect the optical

densities (ODs). Carefully remove any bubbles before performing the reading step.

10. The substrate solution (TMB) is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of cortisol in human serum. The kit is not calibrated for the determination of cortisol in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/ products if false results are suspected.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non- reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method, however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 2-8°C for up to 5 days or at -20°C or lower for up to 30 days.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 20, 50, 100, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

REAGENTS PROVIDED

1. **Anti-Cortisol Antibody-Coated Break-Apart Well Microplate** — Ready To Use
   Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.
   Storage: Refrigerate at 2–8°C
   Stability: Unopened; stable until expiry. Opened; stable for 4 weeks

2. **Cortisol Peroxidase (HRP) Conjugate Concentrate** — Ready to Use
   Contents: Cortisol-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 20 mL bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened; stable until expiry. Opened; stable for 4 weeks

3. **Cortisol Saliva Calibrators** — Ready To Use
   Contents: Six vials containing cortisol in a human serum- based buffer with a non-mercury preservative. Prepared by spiking serum with a defined quantity of cortisol.

   * Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
A	0 µg/dL	1.0 mL
B	0.4 µg/dL	1.0 mL
C	1 µg/dL	1.0 mL
D	4 µg/dL	1.0 mL
E	10 µg/dL	1.0 mL
F	40 µg/dL	1.0 mL

   Storage: Refrigerate at 2–8°C.
   Stability: Unopened; stable until expiry. Opened; stable for 4 weeks

4. **Controls** — Ready to Use
   - Contents: Two vials containing cortisol in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of cortisol. Refer to vial labels for the acceptable range.
   - Volume: 1 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: Unopened; stable until expiry. Opened; stable for 4 weeks

5. **Wash Buffer Concentrate** — Requires Preparation x10
   - Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
   - Volume: 50 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: Unopened; stable until expiry. Opened; stable for 4 weeks. Following preparation, buffer is stable for 2 weeks
   - Preparation: Dilute the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. **TMB Substrate** — Ready To Use
   - Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   - Volume: 16 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: Unopened; stable until expiry. Opened; stable for 4 weeks

7. **Stopping Solution** — Ready To Use
   - Contents: One bottle containing 1M sulfuric acid.
   - Volume: 6 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: Unopened; stable until expiry. Opened; stable for 4 weeks

ASSAY PROCEDURE

Specimen Pretreatment: None

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solution for wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 20 µL of each calibrator, control, and specimen sample into correspondingly labeled wells in duplicate.
4. Pipette 150 µL of the conjugate working solution into each well. (We recommend using a multi- channel pipette.)
5. Swirl plate for 10 seconds and incubate for 45 minutes at room temperature.

6. Wash the wells 3 times with 350 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 150 µL of TMB substrate into each well at timed intervals.
8. Swirl plate for 10 seconds and incubate for 15 minutes at room temperature
9. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 7.
10. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 40 µg/dL, then dilute it with calibrator A at a dilution of no more than 1:10. The result obtained should be multiplied by the dilution factor.

QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated

1. The calibrator A mean optical density meets the acceptable range as stated in the QC certificate included with the kit
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC certificate.

   % Binding=(OD of calibrator/OD of calibrator A) x 100

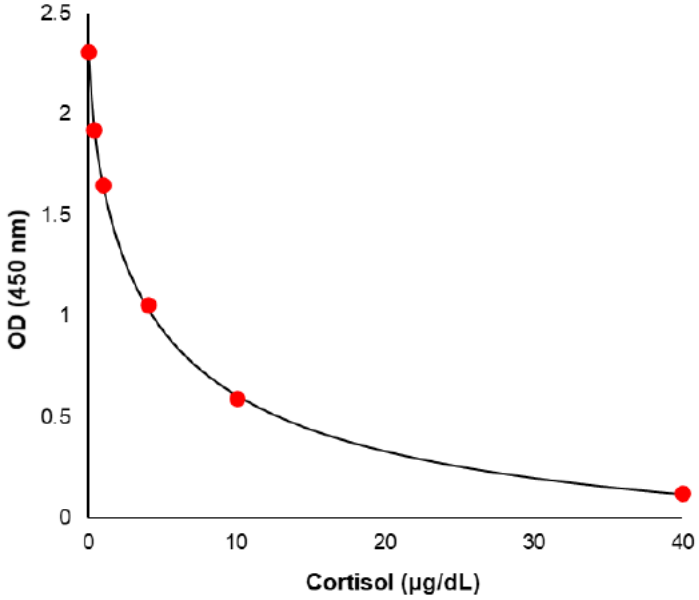
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate
4. The results of any external controls that were used meet the acceptable range

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD	% Binding	Value (µg/dL)
A	2.307	100	0
B	1.926	84	0.4
C	1.651	72	1
D	1.056	46	4
E	0.593	26	10
F	0.123	5	40
Unknown	0.738	-	7.5

TYPICAL CALIBRATOR CURVE



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the DBC Cortisol ELISA kit is 0.4 µg/dL.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Cortisol Saliva ELISA kit with cortisol cross-reacting at 100%.

Steroid	% Cross-Reactivity
Cortisol	100
Prednisolone	13.6
Corticosterone	7.6
Deoxycorticosterone	7.2
Progesterone	7.2
Cortisone	6.2
Deoxycortisol	5.6
Pednisone	5.6
Dexamethasone	1.6

No cross reaction was detected with DHEAS and Tetrahydrocortisone.

Please note that there is an observed cross-reactivity of 13.6% with prednisolone. Since prednisone is converted to prednisolone in vivo, caution must be exercised when assaying the cortisol levels of patients undergoing either therapy.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in µg/dL) are tabulated below:

Sample	Mean	SD	CV %
1	1.44	0.14	9.4
2	14.06	0.41	2.9
3	37.55	1.87	5.0

INTER-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in µg/dL) are tabulated below:

Sample	Mean	SD	CV %
1	1.60	0.13	8.1
2	15.01	0.74	5.0
3	38.18	1.43	3.8

RECOVERY

Spiked samples were prepared by adding defined amounts of cortisol to three patient serum samples (1:1). The results (in µg/dL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	3.86	-	-
+ 2.0	3.61	2.93	123.2
+ 10.0	7.63	6.93	110.1
+ 30.0	16.93	18.72	90.4
2 Unspiked	6.06	-	-
+ 5.0	3.74	3.28	114.0
+ 10.0	9.06	8.03	112.8
+ 60.0	32.49	33.03	98.4
3 Unspiked	10.91	-	-
+ 0.5	6.58	5.70	115.4
+ 5.0	8.74	7.95	109.9
+ 60.0	39.04	35.40	110.3

LINEARITY

Three patient serum samples were diluted with calibrator A.
The results (in µg/dL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	10.94	-	-
1:2	6.13	5.47	112.1
1:4	3.19	2.74	116.4
1:8	1.55	1.37	113.1
2	18.92	-	-
1:2	9.64	9.46	101.9
1:4	4.61	4.73	97.5
1:8	1.98	2.37	83.7
3	42.00	-	-
1:2	20.44	21.00	97.8
1:4	9.57	10.50	94.0
1:8	4.76	5.25	90.7

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Mean (µg/dL)	Mean (µg/dL)
Males and Females – AM	15.59	3.95-27.23
Males and Females – PM	5.93	1.45-10.41

REFERENCES

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.