



EAGLE
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Cortisol ELISA Assay Kit

Catalog Number:

COR31-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

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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 Cortisol ELISA is a competitive immunoassay. Competition occurs between cortisol present in calibrators, controls, specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of anti-cortisol antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-colored product that is Inversely proportional to the amount of cortisol present. Following an Incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the color from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of cortisol in specimen samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For research use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
 1. Do not pipette by mouth.
 2. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
 3. Wear protective clothing and disposable gloves.
 4. Wash hands thoroughly after performing the test.
 5. Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. 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.
4. Do not use this kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.

12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate 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.
15. Do not use grossly hemolyzed, grossly lipemic, icteric, or improperly stored serum.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >40 µg/dL. If further dilution and retesting is required, only Calibrator A may be used to dilute samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of saker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. 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

LIMITATIONS

This kit is intended for research use only and is not to be used for any diagnostic procedures.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

The calibrators and controls provided with the kit contain processed human serum/plasma that has been tested by approved methods and found to be negative for the presence of HBsAg and antibodies to HCV, HIV 1/2 and HIV NAT. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4-5 ml of venous blood into an appropriately labelled tube and allow it to clot. Centrifuge at room temperature and carefully transfer the serum into a new storage tube or container. Serum samples may be stored at room temperature for up to 24 hours, 2-8°C for up to 5 days or at -20°C or lower for up to 30 days. Specimens may be more stable than indicated. 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, 150 and 350 μ L
2. Automatic microplate washer (recommended)
3. Disposable pipette tips
4. Distilled or deionized water
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater

REAGENTS PROVIDED

1. **Anti-Cortisol Polyclonal 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. **Calibrator A-F** — Ready To Use
Contents: Six bottles of calibrator containing specified cortisol concentrations. Human serum-based matrix with a non-mercury preservative. Prepared by spiking matrix with defined quantities of cortisol.

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

Calibrator	Concentration	Volume/Vial
A	0 μ g/dL	1.0 mL
B	0.4 μ g/dL	1.0 mL
C	1 μ g/dL	1.0 mL
D	4 μ g/dL	1.0 mL
E	10 μ g/dL	1.0 mL
F	40 μ g/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 bottles of control containing different cortisol concentrations. Human-serum based matrix with a non-mercury preservative. Prepared by spiking matrix with defined quantities of cortisol. Refer to QC for the target values and acceptable range.
Volume: 1.0 mL/bottle
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 the expiry date printed on the label. After Opening: Stable for four weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2–8°C) when not in use.
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 distilled or deionized 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. After all kit components have reached room temperature, mix gently by inversion.
2. Prepare the Wash Buffer Working Solution
3. Plan the microplate wells to be used for calibrators, controls, and samples. Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
4. Pipette **20 µL** of each calibrator, control, and specimen sample into assigned wells.
5. Pipette **150 µL** of the HRP conjugate into each well. (We recommend using a multi- channel pipette.)

6. Gently tap the microplate frame for 10 seconds to mix the contents of the wells and **incubate** the microplate (no shaking) at room temperature for **45 minutes**.
7. Wash the microplate with an automatic plate washer (preferred) or manually as stated below.
 - **Automatic:** Using an automatic microplate washer, perform a 3-cycle wash using 350 µL /well of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells then filling each well with 350 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.
 - **Manually:** For manual washing, perform a 3-cycle wash using 350 µL /well of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 350 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.
8. Pipette **150 µL** of TMB substrate into each well (the use of a multi-channel pipette is recommended)
9. Gently tap the microplate for 10 seconds to mix the contents of the wells and incubate at room temperature (no shaking) for 15 minutes.
10. Pipette 50 µL of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
11. Measure the optical density (absorbance) In the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

CALCULATIONS

1. Calculate the mean optical density of each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density cause and other calibrator curve.
4. 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

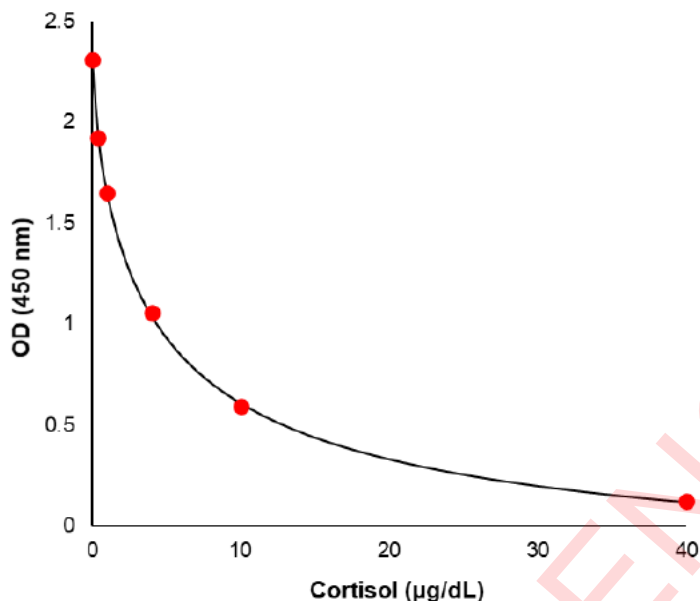
1. The calibrator A mean optical density meets the acceptable range as stated in the QC certificate included with the kit
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC certificate. $\% \text{ Binding} = (\text{OD of calibrator} / \text{OD of calibrator A}) \times 100$
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



Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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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.