EAGLE BIOSCIENCES

Cortisol Saliva ELISA Assay Kit

Catalog Number:
CRT32-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 5 (10 MAY 24)

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INTENDED USE

The Eagle Biosciences Cortisol Saliva ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of Cortisol in human saliva by an enzyme immunoassay. The Eagle Biosciences Cortisol Saliva 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. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a. Do not pipette by mouth.
    b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
    c. Wear protective clothing and disposable gloves.
    d. Wash hands thoroughly after performing the test.
    e. 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 the 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 procedural techniques or pipetting, incomplete washing, or 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 colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, 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 should be reported as > 40 pg/ml and must not be diluted. Dilution will alter the existing equilibrium and 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 the 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 shaker used can influence the optical densities and test results. If a different type of shaker 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

1. This kit is intended for research use only and should not be used for diagnostic procedures.

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 nonreactive 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 1 mL of saliva is required per duplicate determination. Collect 4–5 mL of saliva into a clean glass tube (Salivette by Sarstedt may be used) without force or inducement and before eating, drinking or brushing the teeth. Simply rinse the mouth with water before collection. Do not use blood-contaminated specimens. Store samples at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

Specimen tubes are to be placed into a freezer and allowed to freeze. When ready to use, the specimens are to be thawed and centrifuged. The supernatants are to be collected and poured into freshly labelled tubes.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 50, 150, and 350 µL
2. Disposable pipette tips
3. Automatic microplate washer
4. Distilled or deionized water
5. Benchtop centrifuge
6. Vortex
7. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater
8. Microplate washer (recommended)
9. Polypropylene tubes for sample collection and pre-treatment

REAGENTS PROVIDED

1. **Rabbit 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: 12 months or as indicated on label.

2. **HRP Conjugate Concentrate** — Requires Preparation X50

   Contents: Cortisol-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 450 µL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 440 µL of HRP in 17 mL of assay buffer. Discard any that is left over.

3. **Cortisol Saliva Calibrators** — Ready To Use

   Contents: Six vials containing cortisol in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer 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 ng/mL	2.0 mL
B	0.1 ng/mL	1 mL
C	0.5 ng/mL	1 mL
D	2.5 ng/mL	1 mL
E	10 ng/mL	1 mL
F	50 ng/mL	1 mL

   Storage: Refrigerate at 2–8°C.
   Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. **Controls** — Ready to Use

   Contents: Two vials containing cortisol in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of cortisol. Refer to vial labels for the acceptable range.
   Volume: 1.0 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

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:	12 months or as indicated on label.
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. **Assay Buffer** – Ready to Use

Contents:	One bottle containing a protein-based buffer with a non-mercury preservative.
Volume:	20 mL/bottle
Storage:	Refrigerate at 2–8°C
Stability:	12 months or as indicated on label.

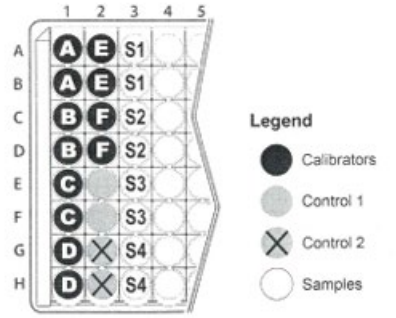
7. **TMB Substrate** — Ready To Use

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:	12 months or as indicated on label.

8. **Stopping Solution** — Ready To Use

Contents:	One bottle containing 1M sulfuric acid.
Volume:	6 mL/bottle
Storage:	Refrigerate at 2–8°C
Stability:	12 months or as indicated on label.

RECOMMENDED LAYOUT



ASSAY PROCEDURE

Specimen Pretreatment: Freezing and Centrifugation

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 solutions of the Cortisol-HRP conjugate and wash buffer.
2. Remove the required number of well strips from the microplate and assemble into a plate frame. Reseal the bag and return any unused strips to the refrigerator.
3. **Pipette 25 µL** of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. **Pipette 150 µL** of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Gently tap the microplate frame for 10 seconds to mix the contents of the wells and **incubate** the microplate at room temperature (no shaking) for **45 minutes.**
6. **Wash** the microplate wells 3 times with working was buffer (350 µL/well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry. The use of a microplate washer is highly recommended. If a microplate washer is not available, ensure that the was buffer reaches the top edge of the wells and that no liquid remains in the microplate after the final washing, avoid splashing.
7. **Pipette 150 µL** of the TMB substrate into each well at timed intervals.
8. Gently tap the microplate frame for 10 seconds to mix the contents of the wells and **incubate** the microplate at room temperature (no shaking) for **15-20 minutes.**
9. **Pipette 50 µL** of stopping solution into each well at the same timed intervals as in step 7.
10. **Measure** the absorbance at 450 nm in all wells with a microplate reader, 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

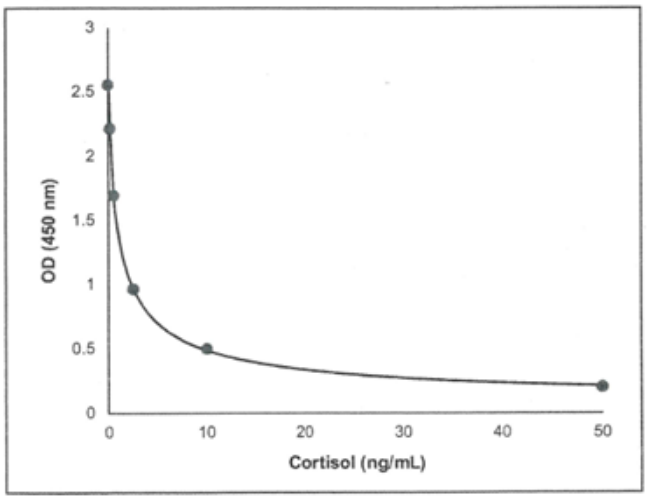
1. Calculate the mean optical density of each calibrator, control, and sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. Read the values of the unknowns directly off the calibrator curve.
4. If a sample reads more than 50 ng/mL dilute it with calibrator A not more than 10-fold. The result obtained must be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD 450nm	% Binding	Value (ng/ML)
A	2.555	100	0
B	2.219	87	0.1
C	1.695	66	0.5
D	0.963	38	2.5
E	0.498	20	10
F	0.204	8	50
Unknown	0.988	-	2.4

TYPICAL CALIBRATOR CURVE



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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.