



EAGLE
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Cortisol LIA Assay Kit

Catalog Number:

COR31-L01 (1 x 96 wells)

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

v. 1.0

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INTENDED USE

The Eagle Biosciences Cortisol LIA Assay Kit is for the direct quantitative determination of cortisol in human serum by a chemiluminescence immunoassay (LIA). The Eagle Biosciences cortisol LIA 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. Measurement of blood cortisol levels 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. Therefore, the free cortisol concentration excreted in the urine is very small, and the 24-hour collection of urine is a must in order to obtain an accurate measurement of urinary cortisol. Cortisol in blood 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 chemiluminescence immunoassay (LIA) test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, control 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 luminescence substrate solution is added. The relative luminescence units (RLUs) are measured on a microtiter plate luminometer. The RLU values are inversely proportional to the concentration of cortisol in the sample. A set of calibrators are 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 or serum pools 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 kit 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. The performance of this assay is markedly influenced by the correct execution of the washing procedure!
9. The luminescence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.



10. When dispensing the substrate, do not use pipettes in which these liquids will come into contact with any metal parts.
11. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
12. 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.
13. 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. This kit is intended for research use only and should not be used in 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 direct contact with reagents. In case of contact, wash with plenty of water.

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 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

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

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 50, 100, 150 and 300 μ L
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate luminometer



REAGENTS PROVIDED AND PREPARATION

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. Cortisol-Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation X100
Contents: Cortisol-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 0.3 mL/vial
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation of conjugate working solution: Dilute conjugate concentrate 1:100 in assay buffer before use (example: 20 µL of conjugate concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 120 µL of conjugate concentrate in 12 mL of assay buffer. Discard any that is left over.

3. Cortisol Calibrators — Ready To Use
Contents: Seven 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 bottle labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 µg/dL	1.0 mL
Calibrator B	0.5 µg/dL	0.3 mL
Calibrator C	2 µg/dL	0.3 mL
Calibrator D	5 µg/dL	0.3 mL
Calibrator E	10 µg/dL	0.3 mL
Calibrator F	30 µg/dL	0.3 mL
Calibrator G	60 µg/dL	0.3 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: One vial containing cortisol in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a defined quantity of cortisol. Refer to vial label for the acceptable range.
Volume: 0.3 mL/vial
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.



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 1:10 in distilled or deionized water before use. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. Assay Buffer
 - Contents: One bottle containing protein-based buffer with a non-mercury preservative.
 - Volume: 15 mL/bottle
 - Storage: Refrigerate at 2–8°C
 - Stability: 12 months or as indicated on label.

7. LIA Substrate Reagent A – Requires Preparation
 - Contents: One vial containing luminol plus enhancer.
 - Volume: 1 mL/vial
 - Storage: Refrigerate at 2–8°C
 - Stability: 12 months or as indicated on label.
 - Preparation: See preparation of LIA working substrate solution.

8. LIA Substrate Reagent B – Requires Preparation
 - Contents: One vial containing stabilized peroxide solution.
 - Volume: 1 mL/vial
 - Storage: Refrigerate at 2–8°C
 - Stability: 12 months or as indicated on label.
 - Preparation: See preparation of LIA working substrate solution.

9. LIA Substrate Reagent C – Requires Preparation
 - Contents: One bottle containing buffer with a non-mercury preservative.
 - Volume: 15 mL/bottle
 - Storage: Refrigerate at 2–8°C
 - Stability: 12 months or as indicated on label.
 - Preparation: See preparation of LIA working substrate solution.

PREPARATION OF LIA WORKING SUBSTRATE SOLUTION

Mix 1 part of LIA substrate reagent A with 1 part of LIA substrate reagent B and dilute this mixture 1:5 with LIA substrate reagent C. This gives the ready to use substrate solution. Prepare fresh for each use.

If the whole plate is to be used prepare working substrate solution as follows:
Combine 1 mL of LIA substrate reagent A with 1 mL of LIA substrate reagent B. To the 2 mL of this mixture add 10 mL of LIA substrate reagent C.

Total volume = 12 mL of working substrate solution.

Stability: Working substrate solution is stable for 24 hours at room temperature.



ASSAY PROCEDURE

Important Notes:

- All reagents must reach room temperature before use.
 - Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.
 - The washing procedure influences the precision markedly; it is essential to ensure the washing is effective and thorough.
1. Prepare working solutions of the conjugate, wash buffer and LIA substrate (refer to reagents provided and preparation section).
 2. Remove the required number of well strips. 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 100 μL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
 5. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
 6. Wash the wells 5 times with 300 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 100 μL of LIA working substrate solution into each well. (We recommend using a multichannel pipette.)
 8. Shake for 5 seconds. Incubate for 10–30 minutes at room temperature without shaking.
 9. Measure the RLUs in each well on a microplate luminometer.

CALCULATIONS

1. Calculate the mean RLU of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean RLUs on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean RLU of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 60 $\mu\text{g}/\text{dL}$ then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA**

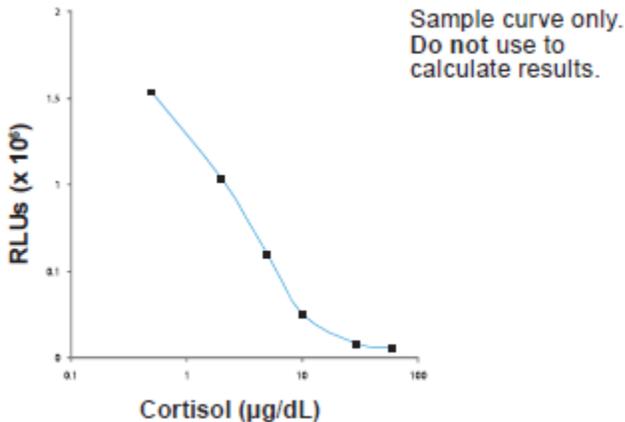
Sample data only. Do not use to calculate results.

Calibrator	RLU 1 $\times 10^3$	RLU 2 $\times 10^3$	Mean RLU $\times 10^3$	RLU/RLU _{MAX} (%)
A, 0 $\mu\text{g}/\text{dL}$	2812	2900	2856	100
B, 0.5 $\mu\text{g}/\text{dL}$	2375	2372	2374	83.1
C, 2 $\mu\text{g}/\text{dL}$	1604	1532	1568	54.9
D, 5 $\mu\text{g}/\text{dL}$	911	854	882	30.9
E, 10 $\mu\text{g}/\text{dL}$	213	220	216	7.6
F, 30 $\mu\text{g}/\text{dL}$	110	102	106	3.7
G, 60 $\mu\text{g}/\text{dL}$	91	87	89	3.1

** It is recommended to use the RLU/RLU_{MAX} values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show quite different RLU values, however, the RLU/RLU_{MAX} values remain consistent.



TYPICAL CALIBRATOR CURVE



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean RLU of calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the cortisol LIA kit is **0.15 µg/dL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the cortisol LIA 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 sixteen times each on the same calibrator curve. The results (in µg/dL) are tabulated below:

Sample	Mean	SD	CV %
1	1.01	0.10	9.9
2	10.44	0.36	3.4
3	29.60	2.82	9.5



INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of two weeks. The results (in $\mu\text{g/dL}$) are tabulated below:

Sample	Mean	SD	CV %
1	1.39	0.13	9.4
2	15.01	0.74	4.9
3	38.18	2.62	6.9

RECOVERY

Spiked samples were prepared by adding defined amounts of angiotensin-I to three patient plasma samples. The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	5.8	-	-
+ 10 $\mu\text{g/dL}$ standard	7.9	7.9	100.0
+ 30 $\mu\text{g/dL}$ standard	18.3	17.9	102.2
+ 60 $\mu\text{g/dL}$ standard	23.5	32.9	71.4
2 Unspiked	9.6	-	-
+ 2 $\mu\text{g/dL}$ standard	5.7	5.8	98.6
+ 5 $\mu\text{g/dL}$ standard	6.5	7.3	109.9
+ 10 $\mu\text{g/dL}$ standard	10.1	9.8	103.1

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in $\mu\text{g/dL}$) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	6.1	-	-
1:2	2.8	3.1	90.3
1:4	1.6	1.6	100.0
1:8	0.9	0.8	112.5
2	7.6	-	-
1:2	3.6	3.8	94.7
1:4	2.0	1.9	105.3
1:8	1.1	1.0	110.0
3	24.8	-	-
1:2	10.5	12.4	84.7
1:4	5.3	6.2	85.5
1:8	2.8	3.1	90.3

EXPECTED VALUES

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

Group	Mean ($\mu\text{g/dL}$)	Range ($\mu\text{g/dL}$)
Males and Females – AM	15.59	3.95-27.23
Males and Females – PM	5.93	1.45-10.41



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