Corticosterone ELISA Kit

Catalog Number KA0468

96 assays

Version: 19

Intended for research use only

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Introduction

Background

Corticosterone is the adrenal steroid, the major glucocorticoid. Glucocorticoid hormones are also known as corticosteroid hormones and are synthesized mainly in the adrenal cortex; however, more recently the enzymes involved in their synthesis have been found in a variety of cells and tissues, including the heart. The effects of these hormones are mediated via both cytoplasmic mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs), which act as ligand-inducible transcription factor (1). Corticosterone has profound effect on the structure and function of the hippocampus (2, 3). Brain corticosterone action through the glucocorticoid receptor may involve memory sotrage (4). Emotional stress might cause increases in plasma corticosterone (5).

Principle of the Assay

The Corticosterone ELISA kit is designed for detection of Corticosterone in plasma, serum, urine, milk, saliva, and cell culture supernatant. This assay employs a quantitative competitive enzyme immunoassay technique that measures Corticosterone in less than 3 hours. A polyclonal antibody specific for Corticosterone has been pre-coated onto a 96-well microplate with removable strips. Corticosterone in standards and samples is competed with a biotinylated Corticosterone sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

General Information

Materials Supplied

List of component

Component	Amount
Corticosterone Microplate: Polystyrene microplate coated with a polyclonal antibody against Corticosterone.	96 (8x12) wells
Sealing Tapes: Pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.	3 slides
Corticosterone Standard: Corticosterone in a buffered protein base (100 ng/ml).	0.5 ml
Biotinylated Corticosterone: Lyophilized.	1 vial
EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base	20 ml
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant	30 ml
Streptavidin-Peroxidase Conjugate (SP Conjugate, 100x): A 100-fold concentrate	80 µl
Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine	8 ml
Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction	12 ml

Storage Instruction

- ✓ Store components of the kit at 2-8℃ or -20℃ upon arrival up to the expiration date.
- ✓ Store Standard and SP Conjugate at -20℃.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8℃.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 30 days at 2-8℃.
- ✓ Store Biotinylated Protein at 2-8℃ before reconstituting with Diluent and at -20℃ after reconstitution.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel).
- ✓ Deionized or distilled reagent grade water.

Precautions for Use

- ✓ Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated protein, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial before opening and using contents.
- ✓ This kit is for research use only.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acid solution

Assay Protocol

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- EIA Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 30 days at 2-8℃.
- Standard Curve: Allow the standard to warm to room temperature prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (100 ng/ml) 1:4 with EIA Diluent to produce 25, 6.25, 1.563, and 0.391 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20℃ and used within 30 days.

Standard Point	Dilution	[Corticosterone] (ng/ml)
P1	Standard (100 ng/ml)	100.0
P2	1 part P1 + 3 part EIA Diluent	25.00
P3	1 part P2 + 3 part EIA Diluent	6.250
P4	1 part P3 + 3 part EIA Diluent	1.563
P5	1 part P4 + 3 part EIA Diluent	0.391
P6	EIA Diluent	0.000

- Biotinylated Corticosterone (2x): Dilute Biotinylated Corticosterone with 4 ml EIA Diluent to produce a 2-fold stock solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:2 with EIA Diluent. Any remaining solution should be frozen at -20℃ and used within 60 days.
- Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20℃.

Sample Preparation

- Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes. Dilute human plasma 1:10, rat plasma 1:200, and mouse plasma 1:200 into EIA Diluent and assay. The undiluted samples can be stored at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. Dilute human serum 1:10, rat serum 1:200, and mouse serum 1:200 into EIA Diluent and assay. The undiluted samples can be stored at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. The undiluted samples can be stored at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. Dilute human urine 1:10, rat urine 1:20, and mouse urine 1:20 into EIA Diluent and assay. The undiluted samples can be stored at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- Saliva: Collect human saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. The undiluted samples can be stored at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- Milk: Collect human milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. The undiluted samples can be stored at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Assay Procedure

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30℃).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 25 µl of Standard and/or Sample per well, and immediately add 25 µl of Biotinylated Corticosterone to each well (on top of the standard or sample). Cover wells with a sealing tape and incubate for 2 hours at room temperature. Start the timer after the last sample addition.
4. Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
5. Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
6. Wash the microplate as described above.
7. Add 50 µl of Chromogen Substrate per well and incubate for about 12 minutes or until the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
8. Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
9. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Assay Summary

Add 25 µl of standard/samples
and 25 µl of biotinylated protein per well.
Incubate 2 hours.

Wash, then add 50 µl of SP per well.
Incubate 30 minutes.

Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 12 minutes.

Add 50 µl of Stop Solution per well.
Read at 450nm immediately.

Data Analysis

Calculation of Results

- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

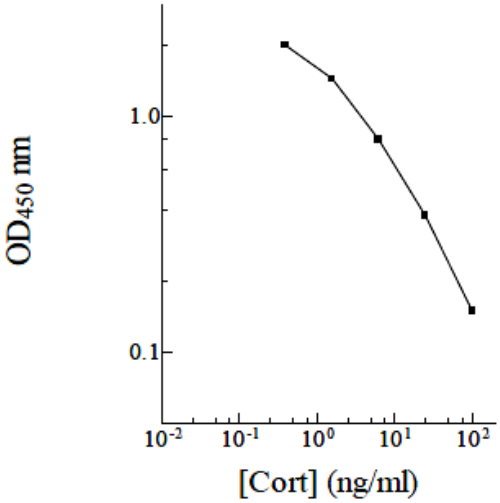

Figure 1: Typical Standard Curve for Corticosterone ELISA Kit

Performance Characteristics

- ✓ The minimum detectable dose of Corticosterone is typically ~0.3 ng/ml.
- ✓ Intra-assay and inter-assay coefficients of variation were 5.0 % and 7.2% respectively.

- Linearity

Average Percentage of Expected Value		
Sample Dilution	Human Plasma	Human Serum
No dilution	107%	106%
1:5	98%	93%
1:10	95%	94%

- Recovery

Standard Added Value	0.5 - 25 ng/ml
Recovery %	84-112%
Average Recovery %	95%

- Reference Values

The normal human plasma levels of Corticosterone are 10-20 ng/ml.

- Cross-Reactivity

Name	% Cross Reactivity
PROGESTERONE	< 2%
ALLOPREGNANOLONE	< 0.1%
CORTEXOLONE	< 1%
DESOXYCORTICOSTERONE	< 30%
CORTISONE	None
CORTEXOLONE HEMISUCCINATE	None
CORTICOSTERONE	100%
6-KETO-17β-ESTRADIOL	None
5-ANDROSTEN-3β-OL-7, 17-DIONE	None
6-KETO-17α-ESTRADIOL	None
3-KETO-5α, 16-ANDROSTENE	None
4-ANDROSTEN-17α-OL-3-ONE	None
ALDOSTERONE	< 2%
ETHYNYLESTRADIOL	None
6-KETOESTRIOL	None
6-KETOESTRONE	None
17β-HYDROXY-4-ANDROSTENE-3, 11-DIONE	< 0.1%
CORTISONE Acetate	None
ALDOSTERONE 21-HEMISUCCINATE	< 0.3%
4-PREGNEN-17, 20β- DIOL-3-ONE	< 0.2%
11α-HYDROXYTESTOSTERONE	None
20α-HYDROXYPROGESTERONE	None
6β-HYDROXYPROGESTERONE	< 0.1%
HYDROCORTISONE	None
17-HYDROXYPROGESTERONE	< 0.1%
CORTISOL	< 0.1%

Resources

References

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Plate Layout

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