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ab108821 Corticosterone ELISA Kit

For the quantitative measurement of Corticosterone in plasma, serum, urine, milk, saliva and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Corticosterone in vitro competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit (ab108821) is designed for the quantitative measurement of Corticosterone levels in plasma, serum, urine, milk, saliva and cell culture supernatant.

A Corticosterone specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently biotinylated Corticosterone is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Conjugate is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is inversely proportional to the amount of Corticosterone captured in plate.

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. Corticosterone has profound effect on the structure and function of the hippocampus. Brain corticosterone action through the glucocorticoid receptor may involve memory storage. Emotional stress might cause increases in plasma corticosterone.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add standard or sample to appropriate wells.

Incubate at room temperature.

Wash and add prepared biotin antibody to each well. Incubate at room temperature.

Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.

Add Chromogen Substrate to each well. Incubate at room temperature

Add Stop Solution to each well. Read immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt, apart from the SP Conjugate & Biotinylated Antibody, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Corticosterone Microplate (12 x 8 wells)	96 wells	4°C
Corticosterone Standard	1 vial	4°C
10X Diluent M Concentrate	20 mL	4°C
Biotinylated Corticosterone Protein (Lyophilized)	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	8 mL	4°C
Stop Solution	12 mL	4°C
20X Wash Buffer Concentrate	30 mL	4°C
Sealing Tapes	3	N/A

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 6 tubes to prepare standard or sample dilutions.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.
- If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

9.1 1X Diluent M

Dilute the 10X Diluent M Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. Store for up to 1 month at 4°C.

9.2 1X Wash Buffer

Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

9.3 1X Biotinylated Corticosterone Protein

9.3.1 Add 4 mL 1X Diluent M to the lyophilized Biotinylated Corticosterone Protein vial to generate a 3X Biotinylated Corticosterone stock solution.

9.3.2 Allow the vial of 3X Biotinylated Corticosterone stock solution to sit for 10 minutes with gentle agitation prior to use.

9.3.3 The stock solution should be further diluted 1:3 with 1X Diluent M to generate 1X Biotinylated Corticosterone.

Δ Note: *Any remaining stock solution should be frozen at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.*

9.4 1X SP Conjugate

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent M.

Δ Note: *The undiluted conjugate should be stored at -20°C.*

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Corticosterone Standard vial at 100 ng/ml corresponds to **Standard #1**.

10.2 Allow the **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

10.3 Label five tubes #2 – 6.

10.4 Add 360 µL of 1X Diluent M to tube #2 – 6.

10.5 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

10.6 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

10.7 Using the table below as a guide, prepare subsequent serial dilutions. 1X Diluent M serves as the zero standard (0 ng/mL) (tube #6).

Standard #	Volume to Dilute (µL)	Volume Diluent M (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Step 10.1				100.0
2	120	360	480	100.0	25.00
3	120	360	480	25.00	6.250
4	120	360	480	6.250	1.563
5	120	360	480	1.563	0.391
6	-	360	360	-	0

11. Sample Preparation

11.1 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. A 4-fold human plasma sample dilution is suggested into EIA Diluent or within the range of 1x-20x; however, user should determine optimal dilution factor depending on application needs. A 100-fold rat or mouse plasma sample dilution is suggested into 1X Diluent M; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

11.2 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. A 4-fold human serum sample dilution is suggested into EIA Diluent or within the range of 1x-20x; however, user should determine optimal dilution factor depending on application needs. A 100-fold rat or mouse serum sample dilution is suggested into 1X Diluent M; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris and collect supernatants. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 Urine:

Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. A 10-fold human urine sample dilution is suggested into 1X Diluent M; however, user should determine optimal dilution factor depending on application needs. A 20-fold rat or mouse urine sample dilution is suggested into EIA Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.5 Saliva:

Collect human saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.6 Milk:

Collect human milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections. The assay is performed at room temperature (18-25°C).

13.2 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.

13.3 Add 25 µl of Corticosterone Standard to each well, and immediately add 25 µl of Biotinylated Corticosterone Protein to each well (on top of the standard). Gently tap plate to ensure thorough mixing. thorough mixing. Break any bubbles that may have formed. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

13.4 Wash five times with 200 µL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4 5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of 1X Wash Buffer and then

invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.

13.5 Add 50 µl of 1X SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

13.6 Wash microplate as described above.

13.7 Add 50 µl of Chromogen Substrate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 20 minutes or until the optimal blue color density develops.

13.8 Add 50 µl of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.

13.9 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 15 minutes, which will reduce the readings.

14. Calculations

Calculate the mean value of the triplicate readings for each standard and sample. To generate a 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 log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

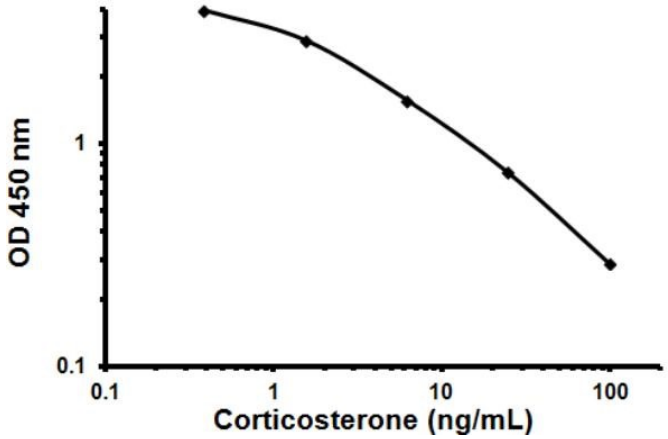

Figure 1. Example of Corticosterone standard curve. The standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The calculated minimum detectable dose is 0.28 ng/mL corticosterone; this is calculated as 2SD from the mean of a zero standard.

The average corticosterone level as measured in human plasma and serum samples from healthy adults (n=40) was 8.0 ng/mL.

PRECISION –

	Intra-assay Precision	Inter-Assay Precision
CV (%)	5.3	10.6

RECOVERY –

Standard Added Value	1.5 - 25 ng/ml
Recovery (%)	84-114 %
Average Recovery (%)	101 %

REFERENCE VALUE -

The normal human plasma levels of Corticosterone are 2 – 22 ng/mL.

LINEARITY OF DILUTION -

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Dilution Factor	Plasma	Serum
1:2	92	94
1:4	101	99
1:8	96	105

17. Assay Specificity

This kit recognizes Corticosterone in plasma, serum, urine, milk, saliva and cell culture supernatants.

Name	% Cross Reactivity
Progesterone	< 2
Allopregnanolone	< 0.1
Cortexolone	< 1
Deoxycorticosterone	< 30
Cortisone	None
Cortexolone Hemisuccinate	None
Corticosterone	100
6-Keto-17β-Estradiol	None
5-Androsten-3β-ol-7, 17-Dione	None
6-Keto-17a-Estradiol	None
3-Keto-5a, 16-Androstene	None
4-Androsten-17a-ol-3-One	None
Aldosterone	< 2
Ethynylestradiol	None
6-Ketoestriol	None
6-Ketoestroe	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
11a-Hydroxytestosterone	None
20a-Hydroxyprogesterone	None
6β-Hydroxyprogesterone	< 0.1

Hydrocortisone	None
17-Hydroxyprogesterone	< 0.1
Cortisol	< 0.1

18. Species Reactivity

This kit recognizes Corticosterone.

Species	Cross Reactivity (%)
Dog	< 50
Cow	None
Monkey	< 50
Mouse	100
Rat	100
Pig	100
Rabbit	100
Human	100

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4°C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly

Problem	Cause	Solution
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)

20. Notes

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