Corticosterone ELISA Kit

Catalog Number KA0468

96 assays

Version: 43

Intended for research use only

www.abnova.com

Abnova

Table of Contents

Introduction **3**

Background 3

Principle of the Assay 3

General Information **4**

Materials Supplied 4

Storage Instruction 4

Materials Required but Not Supplied 4

Precautions for Use 5

Assay Protocol **6**

Reagent Preparation 6

Sample Preparation 6

Assay Procedure 8

Data Analysis **10**

Calculation of Results 10

Performance Characteristics 11

Resources **13**

Troubleshooting 13

References 14

Plate Layout 15

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 cytoplasmic mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs), which act as ligand-inducible transcription factors (1). Corticosterone has a profound effect on the structure and function of the hippocampus (2-3). Brain corticosterone action through the glucocorticoid receptor may involve memory storage (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, milk, urine, saliva, and cell culture samples. This assay employs a quantitative competitive enzyme immunoassay technique that measures corticosterone in approximately 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 steroid sandwiched by the immobilized antibody and streptavidin-peroxidase (SP) conjugate. All unbound material is 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: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against corticosterone.	96 (8x12) wells
Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slides
Corticosterone Standard: Corticosterone in a buffered protein base (100 ng/mL, liquid).	0.5 mL
Biotinylated Corticosterone Steroid (1x): Lyophilized.	1 vial
SBS Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	20 mL
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 mL
SP Conjugate (100x): A 100-fold concentrate.	80 µL
Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 mL
Stop Solution (1x): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction.	12 mL

Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store Standard and SP Conjugate at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ 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.
- ✓ Store Biotinylated Steroid at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

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

- ✓ This product is for Research Use Only and is not intended for use in diagnostic procedures.
- ✓ Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated steroid, 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 insert. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and standard vial before opening and using contents.
- ✓ The Stop Solution is an acidic solution
- ✓ The kit should not be used beyond the expiration date.

Assay Protocol

Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use.
- ✓ SBS Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the SBS Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.
- ✓ Corticosterone Standard: Allow the vial to warm to room temperature with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (100 ng/mL) 4-fold with SBS Diluent to produce 25, 6.25, 1.563, and 0.391 ng/mL solutions. SBS Diluent serves as the zero standard (0 ng/mL). Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C.

Standard Point	Dilution	[Corticosterone] (ng/mL)
P1	1 part Standard (100 ng/mL)	100
P2	1 part P1 + 3 parts SBS Diluent	25
P3	1 part P2 + 3 parts SBS Diluent	6.25
P4	1 part P3 + 3 parts SBS Diluent	1.563
P5	1 part P4 + 3 parts SBS Diluent	0.391
P6	SBS Diluent	0.000

- ✓ Biotinylated Corticosterone Steroid (1x): Reconstitute the Biotinylated Corticosterone Steroid with 3 mL of SBS Diluent to generate a stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.
- ✓ Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with SBS Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

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 and collect plasma. A 4-fold human plasma sample dilution is suggested into SBS Diluent or within the range of 1x-20x; however, user should determine optimal dilution factor depending on application needs. EDTA or Heparin can also be used as an anticoagulant. A 100-fold rat or mouse plasma sample dilution is suggested into SBS 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.

- ✓ 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 SBS 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 SBS 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.
- ✓ Milk: Collect human milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. The sample is suggested for use at 1x; 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.
- ✓ 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 SBS Diluent; however, user should determine optimal dilution factor depending on application needs. A 20-fold rat or mouse urine sample dilution is suggested into SBS 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.
- ✓ Saliva: Collect human saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. The sample is suggested for use at 1x; 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.
- ✓ Cell Culture Supernatants: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatants. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

✓ 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
A) 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 buffer (100x) C) 24 µL of B : 216 µL buffer (10x) = 100000 fold dilution Assuming the needed volume is less than or equal to 240 µL.

Assay Procedure

1. Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
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 Corticosterone Standard or sample to each well, and immediately add 25 µL of Biotinylated Corticosterone Steroid to each well (on top of the standard or sample). Gently tap plate to ensure thorough mixing. 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.
4. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µL of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µL of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µL of 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.
6. Wash the microplate as described above.
7. Add 50 µL of Chromogen Substrate to each 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.

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.
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 low concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

✓ Assay Summary

1. Add 25 μL of Standard or Sample and 25 μL of Biotinylated Steroid per well. Incubate 2 hours.
2. Wash, then add 50 μL of SP Conjugate per well. Incubate 30 minutes.
3. Wash, then add 50 μL of Chromogen Substrate per well. Incubate 20 minutes.
4. Add 50 μL of Stop Solution per well. Read at 450 nm immediately.

Data Analysis

Calculation of Results

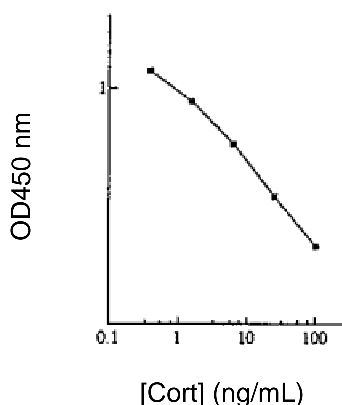
- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression 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.
- ✓ Typical Data

  The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/mL	OD	Average OD
P1	100	0.097 0.093	0.095
P2	25	0.205 0.195	0.200
P3	6.25	0.446 0.430	0.438
P4	1.563	0.841 0.813	0.827
P5	0.391	1.327 1.285	1.306
P6	0.000	1.914 1.858	1.886
Sample: Pooled Normal Sodium Citrate Human Plasma (4x)		0.953 0.941	0.947

- ✓ Standard curve

  The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



- ✓ Reference Value

  Normal human plasma levels of corticosterone range from 3-20 ng/mL.

Performance Characteristics

- ✓ The minimum detectable dose of corticosterone as calculated by 2SD from the mean of a zero standard was established to be 0.28 ng/mL.
- ✓ Intra-assay precision was determined by testing three human plasma samples twenty times in one assay.
- ✓ Inter-assay precision was determined by testing three human plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	6.3%	5.5%	5.7%	10.5%	9.8%	10.2%
Average CV (%)	5.8%			10.2%		

- ✓ Spiking Recovery

  Recovery was determined by spiking two human plasma samples with different corticosterone concentrations.

Sample	Unspiked Sample (ng/mL)	Spiked Sample (ng/mL)	Expected	Observed	Recovery (%)
1	1.0	50	51.00	54.6	107%
		12.5	13.50	14.21	105%
		3.125	4.125	4.026	98%
2	10.0	50	60.00	58.11	97%
		12.5	22.50	21.68	96%
		3.125	13.125	13.48	103%
Average Recovery (%)					101%

✓ Linearity

Human plasma and serum samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
2x	92%	94%
4x	107%	101%
8x	99%	107%

✓ Cross-Reactivity

Steroid	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-17α-ESTRADIOL	None
3-KETO-5α, 16-ANDROSTENE	None
4-ANDROSTEN-17α-OL-3-ONE	None
ALDOSTERONE	None
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

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are empty after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Step performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.

Deficient Standard Curve Fit	Non-optimal sample dilution	• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. • Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. • User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

References

1. Sheppard KE. (2003) Vitam Horm 66:77-112
2. Schaaf MJ et. al. (2000) Stress 3(3):201-8
3. Herbert J. (1998) Exp Gerontol 33(7-8): 713-27
4. Sandi C. (1998) Neural Plast 6(3): 41-52
5. Tanaka M. (1999) Ind Health 37(2): 143-56

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								