AB108821 - Corticosterone ELISA Kit

For the quantitative measurement of Corticosterone in plasma, serum, milk, urine, and saliva samples.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab108821 (use www.abcam.cn/ab108821 for China, or www.abcam.co.jp/ab108821 for Japan)

Storage and Stability
−   Store kit at +4°C immediately upon receipt, apart from the SP Conjugate and Corticosterone Standard, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
−   Alternatively, the entire kit may be stored at -20°C for long term storage before reconstitution - Avoid repeated freeze-thaw cycles.

Materials Supplied

Item	Quantity	Storage Condition
Corticosterone Microplate (12 x 8 wells)	96 wells	4°C
Corticosterone Standard	1 vial	-20°C
10X Diluent S Concentrate	20 mL	4°C
Biotinylated Corticosterone Protein (Lyophilized)	1 vial	4°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 μL	-20°C
Chromogen Substrate	7 mL	4°C
Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	30 mL	4°C
Sealing Tapes	3	N/A

Materials Required, Not Supplied
These materials are not included, but will be required to successfully perform this assay:
−   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.
−   Deionized or distilled reagent grade water.
−   Log-log graph paper or computer and software for ELISA data analysis.
−   6 tubes to prepare standard or sample dilutions.

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.

Δ Note: Concentration of the kit components are lot-specific and the end user should always refer to the vial label.

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

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

2X Biotinylated Corticosterone Protein: Reconstitute the Biotinylated Corticosterone Protein with 3 ml of 1X Diluent S to generate a stock solution. From the stock solution, dilute 2-fold with 1X Diluent S to produce a 1X working solution.
Δ Note: Any remaining stock solution should be frozen at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

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

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).
1.1   Corticosterone Standard vial at 100 ng/ml corresponds to Standard #1.
1.2   Allow the Standard #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
1.3   Label five tubes #2 – 6.
1.4   Add 360 μL of 1X Diluent S to tube #2 – 6.
1.5   To prepare Standard #2, add 120 μL of the Standard #1 into tube #2 and mix gently.
1.6   To prepare Standard #3, add 120 μL of the Standard #2 into tube #3 and mix gently.
1.7   Using the table below as a guide, prepare subsequent serial dilutions. 1X Diluent S serves as the zero standard (0 ng/mL) (tube #6).

Standard #	Volume to Dilute (μL)	Volume Diluent S (μL)	Total Volume (μL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Step 1.1				100
2	120	360	480	100	25
3	120	360	480	25	6.25
4	120	360	480	6.25	1.563
5	120	360	480	1.563	0.391
6	-	360	360	-	0

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 2-fold human, 2-fold mouse, or 4-fold rat plasma sample dilution is suggested into 1X Diluent S; 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).

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 2-fold human, 2-fold mouse, or 4-fold rat serum sample dilution is suggested into 1X Diluent S; 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 4-fold human, 20-fold mouse, or 20-fold rat urine sample dilution is suggested into 1X Diluent S or within the range of 1X – 80X; however, user should determine optimal dilution factor depending on application needs. A 20-fold rat or mouse urine sample dilution is suggested into 1X Diluent S; 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 or within the range of 2X – 10X into 1X Diluent S; 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 or within the range of 2X – 10X into 1X Diluent S; 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.

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*

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.

Assay Procedure

1. Prepare all reagents, working standards, and samples as directed in the previous sections. The assay is performed at room temperature (18-25°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 Protein to each well (on top of the standard or sample). Gently tap plate to ensure 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.
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.
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 25 minutes. Turn on the microplate reader and set up the program in advance.
6. Wash microplate as described above.
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 in ambient light for 30 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 high concentration points after stopping the reaction for about 15 minutes, which will reduce the readings.

Calculation

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.

Typical Sample Values

SENSITIVITY

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.

PRECISION

	Intra-assay Precision	**Inter-Assay Precision**
CV (%)	5.4	10.6

RECOVERY

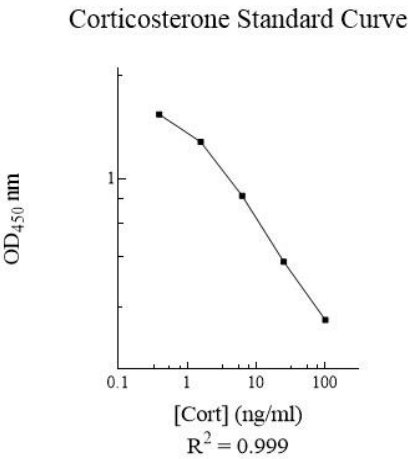| Standard Added Value	0.391 - 25 ng/ml
Recovery (%)	86-112 %
Average Recovery (%)	98 %

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	Human Plasma	Human Serum
1:1	90	90
1:2	94	92
1:4	109	110

STANDARD CURVE

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

Corticosterone Standard Curve

$R^2 = 0.999$

Allopregnanolone	<1%
Cortexolone	<1%
Desoxycorticosterone	None
Cortisone	<0.5%
6-Keto-17β-Estradiol	<0.1%
5-Androsten-3β-OL-7, 17-Dione	<0.1%
6-Keto-17a-Estradiol	<0.1%
3-Keto-5a, 16-Androstene	None
4-Androsten-17a-OL-3-One	<0.1%
Aldosterone	<0.3%
Ethynyl Estradiol	None
6-Ketoestriol	None
6-Ketoesterone	<0.1%
17β-Hydroxy-4-Androstene-3, 11-Dione	<0.1%
4-Pregnen-17, 20β-Diol-3-One	<0.1%
11a-Hydroxytestosterone	None
20a-Hydroxyprogesterone	None
6β-Hydroxyprogesterone	None
17-Hydroxyprogesterone	None
Cortisol	<0.1%
Testosterone	<0.1%

CROSS REACTIVITY

This kit is validated for use with **canine, bovine, equine, human, mouse, rat, swine,** and **rabbit** samples

Steroid	Cross-Reactivity (%)
DHT	None
Progesterone	None

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2   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
	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)

5.6

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)