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Cortisol ELISA Kit

A competitive immunoenzymatic assay for the quantitative measurement of Cortisol in saliva.

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

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1. Overview

Abcam's Cortisol in vitro competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Cortisol in saliva.

A 96-well plate has been precoated with anti-Cortisol IgG. Samples and the Cortisol-HRP conjugate are added to the wells, where Cortisol in the sample competes with the added Cortisol-HRP for antibody binding. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is inversely proportional to the amount of Cortisol in the sample and the intensity is measured at 450 nm.

Cortisol is a steroid hormone released from the adrenal cortex in response to a hormone called ACTH (produced by the pituitary gland), it is involved in the response to stress; it increases blood pressure, blood sugar levels, may cause infertility in women, and suppresses the immune system.

Cortisol acts through specific intracellular receptors and has effects in numerous physiologic systems, including immune function, glucose-counter regulation, vascular tone, substrate utilization and bone metabolism. Cortisol is excreted primarily in urine in an unbound (free) form.

The majority of cortisol in saliva is not-bound and enters the saliva via intracellular mechanisms. Salivary cortisol levels are unaffected by salivary flow rate or salivary enzymes.

There is a high correlation between serum and saliva cortisol levels

These normal endogenous functions are the basis for the physiological consequences of chronic stress - prolonged cortisol secretion causes muscle wastage, hyperglycaemia, and suppresses immune/inflammatory responses. The same consequences arise from long-term use of glucocorticoid drugs.

2. Protocol Summary

Prepare all reagents, samples, controls and standards as instructed.



Add samples, standards and controls to wells used.



Add prepared labeled HRP-Conjugate to each well. Incubate at 37°C.



After washing, add TMB substrate solution 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.
- 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.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website <https://www.abcam.com/en-us>

4. Storage and Stability

Store kit at +4°C immediately upon receipt.

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
Anti-Cortisol IgG Coated Microplate (12x8 wells)	96 wells	4°C
Stop Solution	15 mL	4°C
Cortisol-HRP Conjugate (Concentrated)	1 mL	4°C
Incubation Buffer	30 mL	4°C
TMB Substrate Solution	15 mL	4°C
10X Washing Solution	50 mL	4°C
Control A*	1 mL	4°C
Control B*	1 mL	4°C
Cortisol Standard 0 – 0 ng/mL	1 mL	4°C
Cortisol Standard 1 – 0.5 ng/mL	1 mL	4°C
Cortisol Standard 2 – 1 ng/mL	1 mL	4°C
Cortisol Standard 3 – 5 ng/mL	1 mL	4°C
Cortisol Standard 4 – 10 ng/mL	1 mL	4°C
Cortisol Standard 5 – 20 ng/mL	1 mL	4°C
Cortisol Standard 6 – 100 ng/mL	1 mL	4°C
Foil Cover	1 unit	4°C

*The controls reflect typical samples taken in the morning (control B) and in the evening (control A).

The target values for these controls are:

Control A: ~ 1 – 2.4 ng/ml

Control B: ~ 5.5 – 11.2 ng/ml

They can be used as reference control for different day times

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer

8. Technical Hints

- 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.
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- **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.**

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.

9.1 1X Washing Solution

Prepare 1X Washing Solution by diluting 10X Washing Solution with deionized water. To make 500 mL 1X Washing Solution combine 50 mL 10X Washing Solution with 450 mL deionized water. Mix thoroughly and gently.

9.2 1X Cortisol-HRP Conjugate

Add 10 µL Cortisol-HRP Conjugate (Concentrated) solution to 1 mL Incubation Buffer. Mix thoroughly and gently for 5 minutes on a rotating mixer. The 1X Cortisol-HRP Conjugate should be prepared immediately before use. If required however, the 1X Cortisol-HRP Conjugate is stable for 3 hours at room temperature.

- All solutions are supplied ready to use
- Control A and B are light sensitive – should be kept in dark.

10. Sample Preparation

- The determination of Cortisol can be performed in saliva. It is recommended to collect saliva samples with a centrifuge glass tube and a plastic straw or with the “Salivette”.
- Avoid repeated freezing and thawing.
- The Saliva samples should remain undiluted.

Refer to Dilution Guidelines for further instruction.

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

Consideration for the samples collection

- a) It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other extraneous materials.
- b) If saliva collection is carried out in the morning, ensure that this is carried out prior to brushing teeth.
- c) If saliva collection is carried out during the day, allow 1 hour after any food, drink or medication intake before collecting saliva samples.
- d) If rinsing the mouth with water, allow 10 min before collection.

Collection instructions using centrifuge glass tube and a plastic straw:

1. Let the saliva flow down through the straw into the centrifuge glass tube.
2. Centrifuge at 3000 rpm for 15 min.
3. Store at -20°C for at least 1 hour.
4. Defrost samples.
5. Centrifuge at 3000 rpm for 15 min.
6. The saliva sample is now ready to be tested.
7. Store the sample at 2 to 8 °C for one week or at -20°C for longer time.

The kit is also compatible with Sarstedt Salivette® collection method, following supplier instructions.

11. 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 strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.
- If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 μ L to 350 μ L to avoid washing effects.
- Assay all standards, controls and samples in duplicate.

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

12.3 Add 25 μ L standards and samples into their respective wells. Add 200 μ L 1X Cortisol-HRP Conjugate to each well. Leave a blank well for substrate blank.

12.4 Cover wells with the foil supplied in the kit.

12.5 Incubate for 1 hour at 37°C.

12.6 When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 μ L diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be > 5 seconds. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step.

ΔNote: Washing is critical. Insufficient washing results in poor precision and falsely elevated absorbance values.

12.7 Add 100 μ L TMB Substrate Solution into all wells.

12.8 Incubate for exactly 15 minutes at room temperature in the dark.

12.9 Add 100 μ L Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.

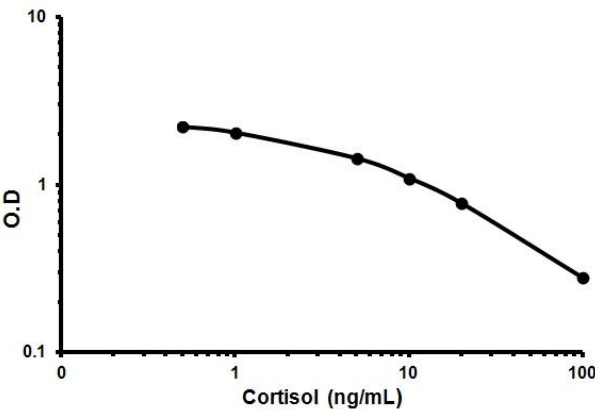
12.10 Measure the absorbance of the sample at 450 nm within 30 minutes of addition of the Stop Solution.

13. Calculations

- Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).
- Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

14. Typical Data

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



Conc. (ng/mL)	O.D
0.0	2.45
0.5	2.22
1.0	2.05
5.0	1.44
10.0	1.10
20.0	0.78
100.0	0.28

Figure 1. Example of Cortisol standard curve.

15. Typical Sample Values

REFERENCE VALUE –

The following values can be used as preliminary guideline until each laboratory established its own normal ranges.

A.M. 3.0 – 10.0 ng/ml

P.M. 0.6 – 2.5 ng/ml

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

SENSITIVITY –

The lowest detectable concentration of Cortisol that can be distinguished from the zero standard is 0.12 ng/mL at the 95 % confidence limit.

PRECISION –

	Intra-Assay	Inter-Assay
n=	20	10
%CV	≤ 10	≤ 8.3

RECOVERY –

The recovery of 6.25 – 12.5 – 25 – 50 ng/ml of Cortisol added to a saliva sample gave an average value ($\pm$ SD) of 95.42% $\pm$ 9.11% with reference to the original concentrations.

16. Assay Specificity

The cross reaction of the antibody calculated at 50% is:

Cortisol	100 %
Prednisolone	46.2 %
11-Deoxycortisol	4 %
Cortisone	3.69 %
Prednisone	3.10 %
11 α OH Progesterone	1 %
Progesterone	< 0.1 %
Aldosterone	< 0.1 %
Pregnenolone	< 0.1 %
17 beta Estradiol	< 0.1 %
Estrone 3-sulfate	< 0.1 %
Estriol	< 0.1 %
Testosterone	< 0.1 %
Spironolactone	< 0.1 %
DHEA	< 0.1 %
DHEA-S	< 0.1 %
Androstenedione	< 0.1 %
Androsterone	< 0.1 %
DHT	< 0.1 %
Danazol	< 0.1 %
Cholesterol	< 0.1 %
Dexamethasone	< 0.1 %

Please contact our Technical Support team for more information.

17.Troubleshooting

Problem	Cause	Solution
Low signal	Incubation time too short	Try overnight incubation at 4 °C
	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 & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. Notes

Technical Support

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