ab108674 – Cortisol ELISA Kit

Instructions for Use

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

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

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1. BACKGROUND

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

A 96-well plate has been precoated with anti-Cortisol. 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) and is involved in the response to stress; increases in blood pressure and blood sugar levels, suppression of the immune system and may cause infertility in women.

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.

Cortisol binds in plasma to corticosteroid-binding globulin (CBG, transcotin), with high affinity, and to albumin. Only free Cortisol is available to most receptors.

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 usage of glucocorticoid drugs.

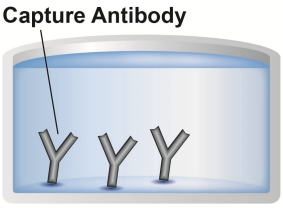
The free Cortisol fraction represents the metabolically active Cortisol. In normal conditions, less than 1% is excreted in urine. In pathological conditions (Cushing's syndrome), the levels of free urinary Cortisol are

elevated, as the CBG does not bind to the Cortisol in the plasma, therefore the excess is removed in urine.

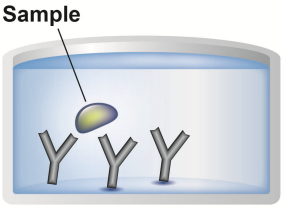
During pregnancy or estro-progestogen treatment an increase of plasma Cortisol is caused by an increase in the production of the transport protein, but the levels of free urinary Cortisol are normal indicating correct adrenal function.

This test is very useful to estimate the real adrenal function, because the dose of free Cortisol is in the metabolically active form. Moreover the measurement of free urinary Cortisol is the better parameter for the diagnosis of the Cushing's syndrome.

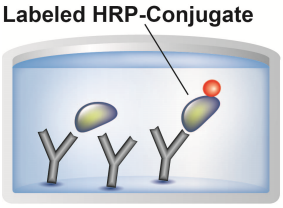
2. ASSAY SUMMARY



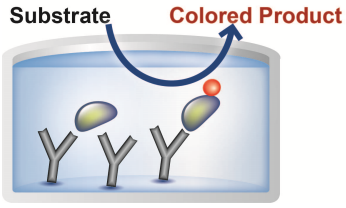
Prepare all reagents, samples, controls and standards as instructed.



Add samples, standards and controls to wells used.



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



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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-Cortisol IgG Coated Microplate (12 x 8 wells)	96 Wells	2-8ºC
Stop Solution	15 mL	2-8ºC
Cortisol-HRP Conjugate	33 mL	2-8ºC
TMB Substrate Solution	15 mL	2-8ºC
10X Wash Solution	50 mL	2-8ºC
Low Control	1 mL	2-8ºC
High Control	1 mL	2-8ºC
Cortisol Standard 0 – 0 ng/mL	4 mL	2-8ºC
Cortisol Standard 1 – 1 ng/mL	1 mL	2-8ºC
Cortisol Standard 2 – 5 ng/mL	1 mL	2-8ºC
Cortisol Standard 3 – 30 ng/mL	1 mL	2-8ºC
Cortisol Standard 4 – 200 ng/mL	1 mL	2-8ºC
Strip Holder	1 Unit	2-8ºC
Cover Foils	1 Unit	2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize 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

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

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, samples and controls to room temperature (18-25°C) prior to use.

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. Diluted solution is stable for 30 days at 2-8°C. In the concentrated solution it is possible to observe the presence of crystals, in this case mix at room temperature until complete dissolution of crystals.

- All other solutions are supplied ready to use

10. SAMPLE COLLECTION AND STORAGE

- The determination of Cortisol with this kit should be performed in urine samples
- The kit has been designed to be used on untreated urine samples; acidification treatments of the urine that lead the pH to values below 5.0 could interfere with the assay and produce aberrant results.
- It is not necessary to dilute the sample.
- The total volume of urine excreted during 24 hours should be collected and mixed in a single container.
- Urine samples which are not to be assayed immediately should be stored at 2…8°C or at – 20°C for longer periods (maximum 6 months)

  Avoid repeated freezing and thawing

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 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**

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

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

13.3. Add 10 µL standards, controls or samples into their respective wells. Add 300 µL Cortisol-HRP Conjugate to each well. Leave a blank well for substrate blank.

13.4. Cover wells with the foil supplied in the kit.

13.5. Incubate for 1 hour at 37°C.

13.6. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL diluted washing 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

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

13.8. Add 100 µL TMB Substrate Solution into all wells.

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

13.10. Add 100 µL 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.

13.11. 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).

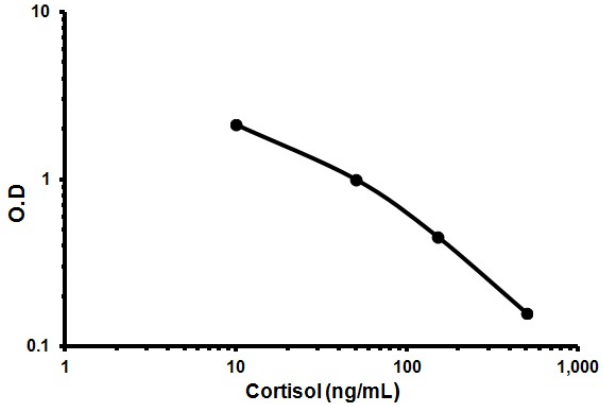
Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

To calculate the Cortisol concentration in urine, calculate as above and correct for total volume of volume of urine collected in 24 hours:

- ng/mL x Vol (mL) urine 24 h/ 1.000 = µg Cortisol/24h

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed. The concentration of standards varies from lot to lot.



Conc. (ng/mL)	O.D
0	2.74
10	2.14
50	1.01
150	0.46
500	0.16

15. TYPICAL SAMPLE VALUES

REFERENCE VALUES-

Human urine Cortisol reference values:

50 – 190 µg/24 hours

SENSITIVITY –

The lowest detectable concentration of Cortisol that can be distinguished from the Standard 0 is 2.95 ng/mL at the 95 % confidence limit.

PRECISION –

	Intra-Assay	Inter-Assay
n=	60	30
%CV	≤ 6.5	≤ 7.2

RECOVERY –

The recovery of 12.5 – 25 – 50 – 100 ng/mL of urinary Cortisol added to a sample gave an average value (±SD) of 107.48% ± 8.16% 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 %
11αOH 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 %

This method allows the determination of Cortisol from 10 – 500 ng/mL.

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to 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

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