ELISA PRODUCT INFORMATION & MANUAL

Human Cortisol ELISA Kit (Colorimetric)

NBP3-18003

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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Intended use

This ELISA kit applies to the in vitro quantitative determination of Cortisol concentrations in Human serum and plasma. Please contact tech-support for other sample type detection.

Specification

- Sensitivity: 2.92ng/mL.
- Detection Range: 6.25-400ng/mL.
- Specificity: This kit recognizes Cortisol in samples. No significant cross-reactivity or interference between Cortisol and analogues was observed.
- Repeatability: Coefficient of variation is <10%.

Background

Cortisol is often called the "stress hormone" because of its connection to the stress response, however, cortisol is much more than just a hormone released during stress. Cortisol is one of the steroid hormones and is made in the adrenal glands. Secretion of the hormone is controlled by the hypothalamus, the pituitary gland, and the adrenal gland, a combination glands often referred to as the HPA axis. Because most bodily cells have cortisol receptors, it affects many different functions in the body. Cortisol can help control blood sugar levels, regulate metabolism, [1] help reduce inflammation, and assist with memory formulation. It has a controlling effect on salt and water balance and helps control blood pressure. In women, cortisol also supports the developing fetus during pregnancy. Low cortisol levels can cause a condition known as primary adrenal insufficiency or Addison disease. While rare, primary adrenal insufficiency is an autoimmune disease that causes damage to the adrenal glands Cortisol reduces bone formation, favoring long-term development of osteoporosis. It transports potassium out of cells in exchange for an equal number of sodiumions. This can trigger the hyperkalemia of metabolic shock from surgery. Cortisol also reduces calcium absorption in the intestine [2].

1. Mooradian, A. D., Morley, J. E., & Korenman, S. G. (1987). Biological actions of androgens. Endocrine reviews, 8(1), 1-28.
2. Myers, J. B., & Meacham, R. B. (2003). Androgen replacement therapy in the aging male. Reviews in urology, 5(4), 216.

Test principle

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with Cortisol. During the reaction, Cortisol in the sample or standard competes with a fixed amount of Cortisol on the solid phase supporter for sites on the Biotinylated Detection Ab specific to Cortisol. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Cortisol in the samples is then determined by comparing the OD of the samples to the standard curve.

Kit components & Storage

An unopened kit can be stored at 4℃ for 1 month. If the kit is not used within 1 month, store the items separately according to the following conditions since the kit is received.

Item	**Specifications**	**Storage**
Micro ELISA Plate (Dismountable)	8 wells ×12 strips	-20℃, 6 months
Reference Standard	2 vials	
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 uL	
Concentrated HRP Conjugate (100×)	1 vial, 120 μL	-20℃(shading light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	4℃, 6 months
Biotinylated Detection Ab Diluent	1 vial, 14 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	4℃(shading light)
Stop Solution	1 vial, 10 mL	4℃
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring.

Other supplies required

Microplate reader with 450 nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
37℃ Incubator
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

Note

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA Plate may appear to have a water-like substance, which is normal and will not have any impact on the experimental results.
3. Do not reuse the diluted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wave length at 450 ±10 nm. The optical density should be within 0~3.5.
5. Do not mix or use components from other lots.
6. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.

Sample collection

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.

Plasma: Collect plasma using EDTA-Na_2 as anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!

Urine: Use a sterile container to collect urine samples. Remove particulates by centrifugation for 15 minutes at 1000×g at 2-8 ℃. Collect the supernatant to carry out the assay.

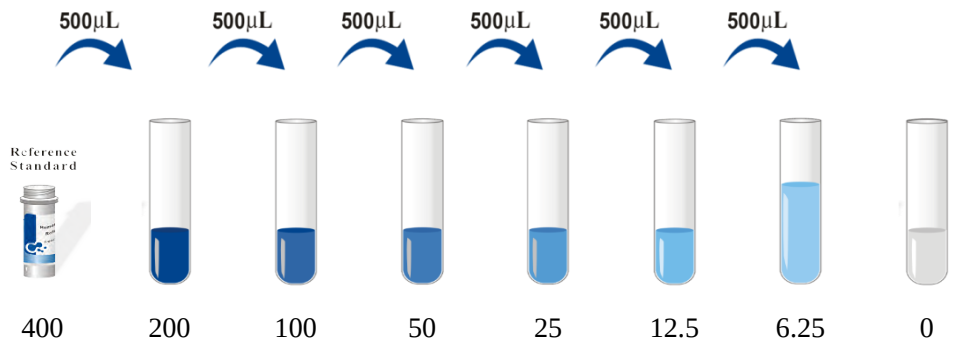
Note for sample:

1. Samples should be assayed within 7 days when stored at 4℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles.
2. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
3. It is recommended to do the experiment with undiluted human serum, plasma and urine samples.

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.
2. **Wash Buffer**: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1min. Add 1.0mL of Reference Standard &Sample Diluent, let it stand for 10min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 400 ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 400, 200, 100, 50, 25, 12.5, 6.25, 0 ng/mL.

   Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 400 ng/mL working solution to the first tube and mix up to produce a 200 ng/mL working solution. Pipette 500uL of the solution from the former tube to the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.



4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (50μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.
5. **Concentrated HRP Conjugate working solution:** Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100×Concentrated HRP Conjugate to 1×working solution with Concentrated HRP Conjugate Diluent.

Assay procedure (A brief assay procedure is on the 11th page**)**

1. Add the **Standard working solution** to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side(50 uL for each well). Add the samples to the other wells(50 uL for each well). Immediately add 50 μL of **Biotinylated Detection Ab working solution** to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37℃.Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Aspirate or decant the solution from each well， add 350 uL of **wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
3. Add 100 μL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37℃.
4. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 2.
5. Add 90 μL of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37℃. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.
6. Add 50 μL of **Stop Solution** to each well. Note: Adding the stop solution should be done in the same order as the substrate solution.
7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation of results

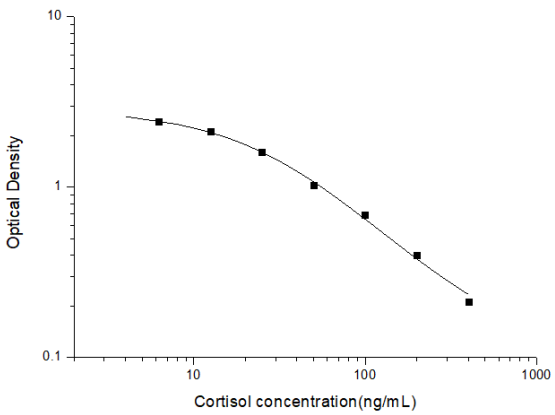
Average the duplicate readings for each standard and samples. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample is under the lowest limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

Concentration(ng/mL)	400	200	100	50	25	12.5	6.25	0
OD	0.212	0.398	0.688	1.024	1.609	2.138	2.429	2.857



Sample values

Samples from Human were evaluated for the presence of Cortisol in this assay.

Sample type	Cortisol concentration range（ng/mL）
Serum(n=10)	63-98
Plasma(EDTA)(n=10)	44-53
Urine(n=5)	19-31

Note: The above values were all from normal healthy samples.

Precision

Intra-assay Precision (Precision within an assay): 3 human samples with low, mid range and high level Cortisol were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 human samples with low, mid range and high level Cortisol were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(ng/mL)	50.33	100.32	200.52	51.27	99.84	202.18
Standard deviation	4.42	8.08	14.90	4.59	7.02	14.46
CV(%)	8.78	8.05	7.43	8.96	7.03	7.15

Recovery

The recovery of Cortisol spiked at three different levels in human samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum(n=5)	81-93	85
EDTA plasma(n=5)	86-96	89
Urine(n=5)	85-100	95

Linearity

Human Samples were spiked with high concentrations of Cortisol and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum(n=5)	Plasma (EDTA)(n=5)	Urine (n=5)
1:2	Range (%)	80-90	88-102	85-107
	Average (%)	85	93	94
1:4	Range (%)	81-96	87-95	80-101
	Average (%)	88	90	92
1:8	Range (%)	84-102	82-89	83-95
	Average (%)	95	86	86
1:16	Range (%)	87-104	89-104	84-99
	Average (%)	93	99	88

Troubleshooting

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells in between steps.
Low signal	Insufficient incubation time	Ensure sufficient incubation time.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation.
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB,rapid coloring.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting onthe Microplate reader.
		Open the Microplate Reader ahead to pre-heat.
Large CV	Inaccurate pipetting	Check pipettes.
High background	Concentration of target protein is too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

SUMMARY

1. Add 50 μL standard or sample to each well. Immediately add 50 μL Biotinylated Detection Ab to each well. Incubate for 45 min at 37℃

2. Aspirate and wash 3 times

3. Add 100 μL HRP Conjugate to each well. Incubate for 30 min at 37℃

4. Aspirate and wash 5 times

5. Add 90 μL Substrate Reagent. Incubate 15 min at 37℃

6. Add 50 μL Stop Solution. Read at 450nm immediately.

7. Calculation of results.

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. The final experimental results will be closely related to the validity of products, operational skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions!
4. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
5. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
6. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipments, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.