Human Cortisol (Cortisol) Competitive ELISA Kit

NB-22-71826

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Cat# NB-22-71826

This ELISA kit used for quantitative determination of Cortisol in human serum, plasma, saliva and urine samples.
We highly recommended reading this manual thoroughly before using this kit.

Introduction

This kit is a competitive enzyme immunoassay (ELISA) for in-vitro quantitative measurement of cortisol in human serum, plasma, saliva, and urine samples.

Principle of The Assay

This ELISA kit uses the Competitive-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with human Cortisol. The samples (or standards) are added, along with an HRP-conjugated antibody which specifically binds human Cortisol. During the reaction Human Cortisol in the sample or standard competes with a fixed amount of human Cortisol on the solid phase supporter for sites on the HRP-conjugated detection antibody specific to human Cortisol. Excess conjugate and unbound sample or standard are washed away. A substrate reagent is then added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the colour turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Cortisol in tested samples can be calculated by comparing the OD of the samples to the standard curve.

Sensitivity: 2.87 ng/mL
Detection Limit: 6.25~400 ng/mL
Assay run time: 1-2 hours

Components

Component	Size (96T)	Storage recommendation
Micro-ELISA Coated Plate	8×12	Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20°C for up to 6 months. Reseal along entire edge of zip-seal.
Standard (Lyophilized)	2 vials	Aliquot and store at ≤ -20 °C for up to 6 months. * Avoid repeated freeze-thaw cycles.
Concentrated HRP- Conjugated Antibody (100×)	1 × 60 µL	May be stored for up to 6 months at -20°C.Protect from light.
Standard/Sample Diluent	1 × 10 mL	
HRP-Conjugated Antibody Diluent	1 × 7 mL	
Wash Buffer (25x)	1 × 30 mL	May be stored for up to 6 months at 2-8°C. (Protect TMB Substrate from light)
Substrate Reagent	1 x 10 mL	
Stop Solution	1 × 10 mL	
Plate Sealers	5 Strips	

Sample Collection and Storage

1. Serum

Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000xg. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

2. Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately, or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

3. Saliva

Centrifuge the sample for 10 minutes at 4000xg 2-8°C to remove any particulates. Collect the supernatant to use in the assay. We recommend using fresh samples for the assay

4. Urine

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

Note: Samples should be assayed within 7 days when stored at 2-8°C, or should be divided up and stored at -20°C (≤1 month) or -80°C (≤3 months), avoiding freeze-thaw cycles. We recommend predicting the concentration required before assaying. If the sample concentration is not within the range of the standard curve the user should determine the optimal sample dilutions for their experiments. If using serum or plasma samples, we recommend not diluting them for this experiment.

Precautions

1. This kit is for RESEARCH USE ONLY.
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Do not reuse the reconstituted standard-HRP-conjugated detection antibody working solution. Unspent antibody and other stock solutions should be stored according to the storage conditions in the above table.
4. Variations in sample collection, processing, and storage may cause sample value differences.
5. Reagents may be harmful. If contact made with skin, rinse with an excess amount of tap water.
6. Stop Solution contains strong acid. Wear eye, hand, and face protection.
7. Do not mix or substitute reagents with those from other lots or other sources.
8. Adequate mixing is very important for a good result. Use a mini-vortex at the lowest frequency.
9. Mix each sample and all components in the kits adequately and use a clean plastic container to prepare diluent.
10. Samples and standards should be assayed in duplicate, and the sequence of the regents should be added consistently.
11. Reuse of the dissolved standard is not recommended.
12. The kit should not be used beyond the expiration date on the kit label.
13. The kit should be kept away from light when it is stored or incubated.
14. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with appropriate regulations.
15. To avoid cross contamination, please use disposable pipette tips.
16. Please prepare all kit components according to the specification. If the kits will be used several times, keep unused strips sealed and preserve with desiccants. Use within 2 months.

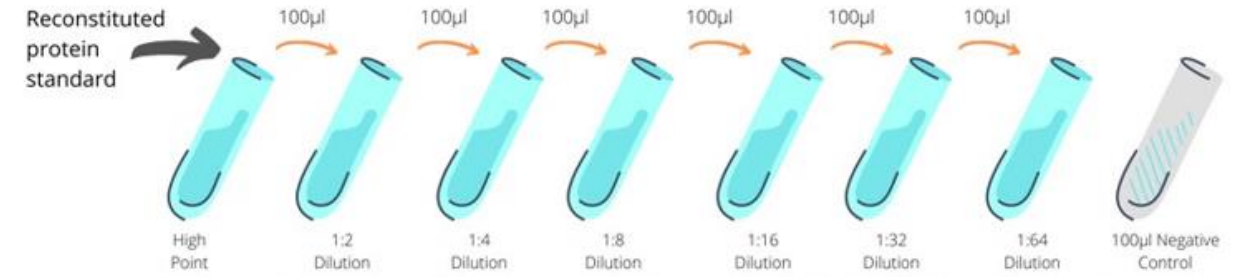
Experiment Materials

The following materials are required to carry out the aforementioned assay but are not included with this kit.

1. Microplate reader (measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm).
2. Pipettes and pipette tips: 0.5-10, 2-20, 20-200, 200-1000 µL.
3. Microplate washer, Squirt bottle.
4. Micro-oscillator.
5. Deionized or double distilled water graduated cylinder.
6. Polypropylene Test tubes for dilution.
7. Incubator capable of maintaining 37°C.

Reagent Preparation

1. Bring all reagents to room temperature before use. If crystals have formed in the concentrate bring it to room temperature and mix gently until the crystals have completely dissolved.
2. Standard: Centrifuge the lyophilised standard at 10,000xg for 1 minute and then add 1mL of the Standard/Sample Diluent to it. Allow the solution to stand for 10 minutes with gentle agitation prior to making further dilutions. Once the protein has fully dissolved the solution should have a concentration of 1000 pg/mL. Prepare a series of EP tubes containing Standard/Sample Diluent, and carry out a serial dilution according to the picture shown below (with recommended concentrations for the standard curve: 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, 15.63 pg/mL). Any remaining standard solution can be aliquoted and stored at -20°C to -70°C.



Two-fold dilution series: Each microvial starts with 100µl diluent. 100µl is carried over from the reconstituted protein standard, thoroughly mixed and then 100µl is transferred to the next. The process is repeated in each microvial in the sequence. No treatment is carried out on the negative control, which should hold 100µl of diluent.

Dilution Method

1. HRP-Conjugated Antibody (100x): Calculate the required amount of antibody needed to perform the experiment in advance. 50µL of working antibody will be needed per well, and we recommend preparing slightly more than needed (as represented in the table below). Centrifuge the concentrated antibody at 800xg for 1 minute and then dilute 1:99 with the HRP-Conjugated Antibody Diluent.

No. of strips	Concentrated HRP-Conjugated Antibody (100x)	HRP-Conjugated Antibody Diluent
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

2. Wash buffer: Dilute 1:24 with double distilled or deionized water before use.

Assay Procedure

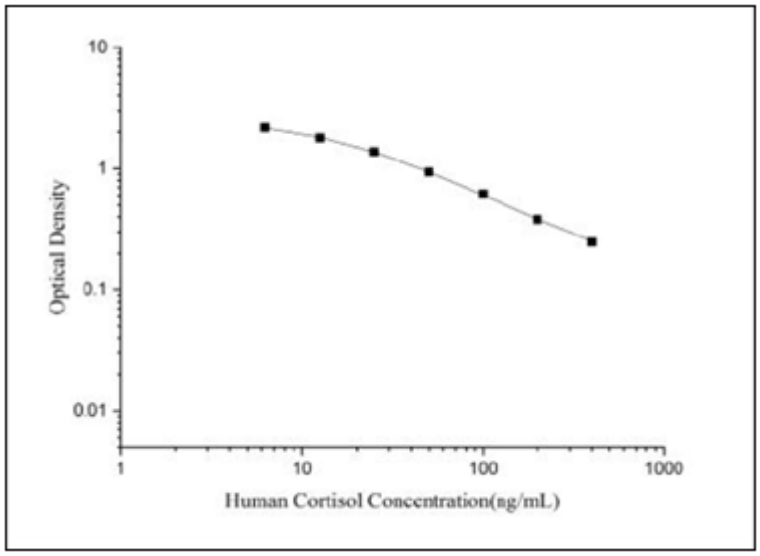
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack and reseal.
2. Add 50 µL of each concentration of standard to their allocated wells, add 50 µL of the samples to their allocated wells, and add 50 µL of Standard/Sample Diluent to the blank well.
3. Immediately add 50 µL of HRP-Conjugated Antibody Working Solution to each of the wells. Cover the plate with the adhesive strip sealer provided and incubate for 60 minutes at 37°C. (Note: solution should be added to the bottom of ELISA plate well, avoid touching the inside wall and foaming.)
4. Decant all the liquid from the plate and add 350 µL wash buffer to each well. Soak for 1 minute and then aspirate. The plate can also be pat dry against clean absorbent paper. Repeat this step 5 times. (A plate washer can also be used for this step). Do not allow the wells to become completely dry.
5. Add 90 µL Substrate Reagent to each well and incubate for 15 minutes at 37°C. Protect from light. Note: The reaction time may be increased or decreased according to the colour change, but do not extend this beyond 30 minutes.
6. Add 50 µL of Stop Solution to each well. Determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
7. Upon completion of the experiment ensure you return unused reagents to their appropriate storage locations.

Calculation of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y- axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the Cortisol concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data

The standard curves are provided for demonstration only. A standard curve should be generated for each set of Cortisol assayed.



Precision

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

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

Inter-plate Precision

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

Recovery

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

Matrix	Recovery range (%)	Average (%)
Serum (n=10)	84-96	90
EDTA plasma (n=10)	90-103	96
Urine (n=10)	91-109	99

Troubleshooting

Problem	Causes	Solutions
Poorly developed standard curve	Inaccurate pipetting.	Check pipetting volume consistency and accuracy.
	Improper standard dilution.	Gently mix the standard solution and dissolve the powder thoroughly in solution.
	Wells were not fully aspirated.	Completely aspirate wells in between stages.
Low fluorescence readings	Insufficient incubation time.	Ensure sufficient incubation time.
	Incorrect assay temperature.	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes or inconsistent dilution.	Check pipettes and ensure correctly prepared.
Large CV	Inaccurate pipetting.	Check pipettes and technique.
High background	Concentration of target protein is too high.	Use recommended dilution factor.
	Plate is insufficiently washed.	Review the manual's washing process. If using a plate washer, check that the ports are not obstructed.
	Contaminated wash buffer.	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the kit.	All the reagents should be stored according to the instructions.
	Too long incubation time.	Ensure precise incubation time.