

Zebrafish Cor (Cortisol) ELISA Kit

Cat: OPK8894

For research use only. Not intended for diagnostic use.

Sensitivity: 1.16 ng/mL

Summary and Explanation

Cortisol (hydrocortisone, compound F) is the most potent glucocorticoid synthesized from cholesterol. Cortisol is found in the blood either as free Cortisol or bound to corticosteroid-binding globulin (CBG). Cortisol production has an ACTH-dependent circadian rhythm with peak levels in the early morning and a nadir at night. The factors controlling this circadian rhythm are not completely defined. Serum levels are highest in the early morning and decrease throughout the day. In the metabolic aspect, Cortisol promotes gluconeogenesis, liver glycogen deposition, and the reduction of glucose utilization. Immunologically, Cortisol functions as an important anti inflammatory, and plays a role in hypersensitivity, immunosuppression, and disease resistance. It has also been shown that plasma Cortisol levels elevate in response to stress. Abnormal Cortisol levels are seen with a variety of different conditions: with adrenal tumors, prostate cancer, depression, and schizophrenia. Elevated Cortisol levels and lack of diurnal variation have been identified in patients with Cushing's disease

Test Principle

Cortisol test kit is a solid phase competitive ELISA. The samples, working Cortisol-HRP Conjugate and anti-cortisol-biotin solution are added to the wells coated with streptavidin. Cortisol in the patient's serum competes with the cortisol enzyme (HRP) conjugate for binding sites. Unbound cortisol and cortisol enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of Cortisol in the samples. A standard curve is prepared relating color intensity to the concentration of the cortisol.

Kit Components

Reagents	96T
Streptavidin coated Micro wells	12stripsx8wells
Cortisol Standard: 6 vials (ready to use)	0.5mL
Cortisol Control: 2 vials(ready to use)	0.5mL
Biotin Reagent: 1 bottle (ready to use)	7mL
Enzyme Conjugate (20X)	0.7mL
Assay Diluent: 1 bottle	12mL
TMB Substrate:1bottle(ready to use)	12mL
StopSolution:1bottle(Ready to use)	12mL
20XWash concentrate: 1bottle	25mL

Storage and Stability

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

Materials Required, Not Supplied

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Safety Notes

1. This kit is only used for lab research and development and should not be used for human or animals.
2. Reagents should be regarded as hazardous substances and should be handled carefully and correctly.
3. Gloves, lab coats, and goggles should always be worn to avoid skin and eyes coming into contact with Stop Reagent and TMB. In case of contact, wash thoroughly with water.

Sample Collection and Storage

Serum - Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4°C, and then centrifuging at 1000×g for 20 minutes. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 1000×g and 2-8°C for 15 minutes 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.

Tissue homogenates -The preparation of tissue homogenates will vary depending upon tissue type.

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince the tissues to small pieces and homogenize them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (PBS can be used as the lysis buffer of most tissues) (w: v=1:9, e.g. 900µL lysis buffer is added in 100 mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000×g and collect the supernatant and assay immediately or store in aliquots at ≤ -20°C.

Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein.

Cell lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10⁷ cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or store in aliquots at ≤ -20°C.

Urine -Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at $1000\times g$ at $2-8^{\circ}\text{C}$ for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Cell culture supernatants and other biological fluids - Centrifuge samples at $1000\times g$ for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Notes

1. Samples to be used within 5 days may be stored at 4°C , otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bio-activity and contamination. Avoid repeated freeze-thaw cycles.
2. Sample hemolysis will influence the result, so it should not be used.
3. When performing the assay, bring samples to room temperature.
4. If the concentration of the test material in your sample is higher than that of the Standard product, please make the appropriate multiple dilutions according to the actual situation (it is recommended to do preliminary experiment to determine the dilution ratio).

Reagent Preparation

1. Cortisol-enzyme Conjugate Solution

Dilute the Cortisol enzyme conjugate 1:21 with assay diluent in a suitable container. For example, dilute 100µL of conjugate with 2mL of assay diluent buffer for 10 wells (A slight excess of solution is made).

2. Wash Buffer

Prepare 1X Wash Buffer by adding the contents of the bottle (25mL, 20X) to 475 mL of distilled or deionized water. Store at room temperature (20-25°C).

Notes

1. After receiving the kit, please store the reagents according to the instructions. The plates can be disassembled to single strips. Please use it in batches on demands. It is recommended that the remaining reagents are used within 1 month after the first test.
2. The test tubes, pipette tips and reagents used in the experiment are all disposable and are strictly prohibited from being reused; otherwise, the experiment results will be affected. Kit reagents of different batches cannot be mixed (except TMB, Washing Buffer and Stop Reagent).
3. Bring all reagents to room temperature (18-25°C) before use. If crystals form in the concentrate (25×), it is a normal phenomenon. Heat it to room temperature (the heating temperature should not exceed 40°C), gently Mix until crystals are completely dissolved.
4. During the washing process, the residual washing liquid in the reaction well should be patted dry on absorbent paper. Do not put the paper directly into the reaction well to absorb water. Before reading, pay attention to remove the residual liquid and fingerprints at the bottom, so as not to affect the microplate reader reading.
5. TMB Substrate Solution is light-sensitive, avoid prolonged exposure to light. Dispense the TMB Substrate Solution within 15 minutes following the washing of the microtiter plate. In addition, avoid contact between TMB Substrate Solution and metal to prevent colour development. TMB is contaminated if it turns blue colour before use and should be discarded. TMB is toxic, avoid direct contact with hands.
6. Bacterial or fungal contamination of either samples or reagents or cross-contamination, between reagents may cause erroneous results

Samples Preparation

1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.
2. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Assay Procedure

Prior to assay, allow reagents to stand at room temperature.

Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Pipette 25 μ L of Cortisol standards, control and patient's sera.
3. Pipette 50 μ L of Biotin reagent to all wells.
4. Add 100 μ L of Cortisol Enzyme Conjugate to all wells
5. Thoroughly mix for 10 seconds.
6. Incubate for 60 minutes at room temperature (20-25°C).
7. Remove liquid from all wells. Wash wells three times with 300 μ L of 1X wash buffer. Blot on absorbent paper towels.
8. Add 100 μ L of TMB substrate to all wells.
9. Incubate for 15 minutes at room temperature (20-25°C).
10. Add 50 μ L of stop solution to all wells. Shake the plate gently to mix the solution.
11. Read absorbance on ELISA Reader at 450 nm within 20 minutes after adding the stop solution.

Calculation of Results

The standard curve is constructed as follows:

1. Check Cortisol standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for Cortisol standards (vertical axis) versus Cortisol standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Concentration(ng/mL)	OD (450 nm)
0	2.77
20	1.42
50	0.79
100	0.43
200	0.22
500	0.12

Precision

Intra-assay Precision (Precision within an assay) : **CV %< 8%**

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) : **CV %< 10%**

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

Declaration

1. The kit may not be suitable for special experimental samples where the validity of the experiment itself is uncertain, such as gene knockout experiments.
2. Certain natural or recombinant proteins, including prokaryotic and eukaryotic recombinant proteins, may not be detected because they do not match the detection antibody and capture antibody used in this product.

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3. This kit is not compared with similar kits from other manufacturers or products with different methods to detect the same object, so inconsistent test results cannot be ruled out.

Analysis of Common Problems and Causes of ELISA Experiment

High background/Non-specific staining

Description of results	Possible reason	Recommendations and precautions
After termination, the whole plate results show a uniform yellow or light colour; or the Standard curve is linear but the background is too high	The yellowing of the whole plate may be caused by wrong addition of other reagents	Check the components and lot numbers of the reagents before the experiment, and confirm that all components belong to the corresponding kit. Reagents from different kits or different lot numbers cannot be mixed.
	ELISA plate was not washed sufficiently	Make sure that the same amount of Washing Solution is added to each microwell during the washing process. After washing, press the ELISA plate firmly on the absorbent paper to remove the residual buffer.
	Incubation time too long	Please strictly follow the steps of the manual
	Streptavidin-HRP contaminates the tip and TMB container or positive control contaminates the Pre-coated Microplate	When absorbing different reagents, the tips should be replaced. When configuring different reagent components, different storage vessels should be used. Please use a pipette during operation.
	Biotinylated-Conjugate or Streptavidin-HRP concentration too high	Check whether the concentration calculation is correct or use after further dilution.
	Substrate exposure or contamination prior to use	Store in the dark at all times before adding substrate.
	Colour development time is too long	Please strictly follow the steps of the manual.
	The wrong filter was used when the absorbance value was read	When TMB is used as the substrate, the absorbance should be read at 450 nm.

No colour plate

Description of results	Possible reason	Recommendations and precautions
After the colour development step, all wells of the ELISA plate are colourless; the positive control is not obvious	Mixed use of component reagents	Please read labels clearly when preparing or using
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the colour developing agent	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3 , etc.), and confirm that the container for preparing the Wash Solution has been washed.
	Missing a reagent or a step	Review the manual in detail and strictly follow the operating steps

Light colour

Description of results	Possible reason	Recommendations and precautions
The Standard is normal, the colour of the sample is light	The sample uses NaN_3 preservative, which inhibits the reaction of the enzyme	Samples cannot use NaN_3
	The sample to be tested may The standard is normal, the colour of the sample is lightnot contain strong positive samples, so the result may be normal	In case of doubt, please test again.
The visual result is normal, but the reading value of the microplate reader is low	Wrong filter used for absorbance reading	When TMB is used as the substrate, the absorbance should be read at 450 nm.

Description of results	Possible reason	Recommendations and precautions
All wells, including Standard and Samples, are lighter in colour	Insufficient incubation time	Timer accurate timing
	Insufficient colour reaction	Usually 15-30minutes
	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements	Reduce the impact of washing, dilute the concentrated lotion and washing time according to the manual, and accurately record the washing times and dosage.
	Distilled water quality problem	The prepared lotion must be tested to see if the pH value is neutral.
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the colour developing agent.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3 , etc.), confirm that the container for preparing the Washing Solution has been washed, and confirm that the purified water for preparing the Washing Solution meets the requirements and is not contaminated.
	The kit has expired or been improperly stored	Please use it within the expiration and store it in accordance with the storage conditions recommended in the manual to avoid contamination.
	Reagents and samples are not equilibrated before use	All reagents and samples should be equilibrated at room temperature for about 30 minutes.
	Insufficient suction volume of the pipette, too fast discharge of pipetting suction, too much liquid hanging on the inner wall of the tip or the inner wall is not clean.	To calibrate the pipette, the tips should be matched, each time the tips should fit tightly, the pipetting should not be too fast, and the discharge should be complete. The inner wall of the tips should be clean, and it is best to use it once.

Description of results	Possible reason	Recommendations and precautions
Poor repeatability	Incubation temperature constant temperature effect is not good	Keep the temperature constant to avoid the local temperature being too high or too low
	When adding liquid, too much remains on the medial wall of wells	When adding liquid, the tip should try to add liquid along the bottom of the medial wall of wells without touching the bottom of the hole.
	Reuse of consumables	The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components.
	The bottom of the microwell is scratched or there is dirt	Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints.
		Technical repetition of the same sample for 3 times, including more than 2 approximate values.
	Cross-contamination during sample addition	Try to avoid cross-contamination when adding samples
The colour of plate is chaotic and irregular	Cross-contamination from manual plate washing	When washing the plates by hand, the first 3 injections of the lotion should be discarded immediately, and the soaking time should be set for the next few times to reduce cross-contamination.
	Cross-contamination when clapping	Use a suitable absorbent paper towel when clapping the plate, do not pat irrelevant substances into the well of the plate, and try not to pat in the same position to avoid cross-contamination.

Description of results	Possible reason	Recommendations and precautions
The colour of plate is chaotic and irregular	The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the colour of plate is chaotic and irregular	Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate and the residual amount should be small when aspirating liquid.
	Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components	Serum plasma should be fully centrifuged at 3000 rpm for more than 6 minutes
	The sample is stored for too long time, resulting in contamination.	Samples should be kept fresh or stored at low temperature to prevent contamination
	Incorrect preparation of Washing Solution or direct misuse of concentrated Washing Solution	Please configure according to the manual

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