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Canine Cortisol(COR) ELISA Kit

Cat.No:CK-bio-18938

Standard Curve Range: 5ng/ml -160ng/ml

Sensitivity: 1.0ng/ml

Expiration date： twelve months .

Storage:2-8℃.

Read this manual carefully before using. The ELISA kit use **competitive assay.**

When stored at 2 -8 °C unopened reagents will retain reactivity until expiration date.

Opened reagents must be stored at 2 -8 °C.

Any further information contact bio@coonkoon.com

OPERATION MANUAL

And the ELISA kits only be used for research purposes, not for medical diagnosis.

Reagent preparation: Bring all reagents to room temperature before using.

For samples: Serum, plasma, cell culture supernatants, body fluid and tissue homogenate.

Intended Use

For the quantitative determination of Canine Cortisol(COR) concentrations in serum, plasma, saliva, urine, tissue homogenate, cell culture supernates,body fluid and other biological fluids.

Test Principle

This kit use competitive method to test the content of Cortisol (COR) in the sample.

Add Standard and Sample to the wells that pre-coated with objective antibody, then add streptavidin HRP to form an immune complex, incubation, by incubation and washing, removal of unbound enzyme, and then add the substrate A and B, then the solution will turn blue and finally change into yellow at the effect of acid.The color depth or light was negatively correlated with the concentration of Cortisol (COR).

MATERIALS PROVIDED WITH THE KIT

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

Reagents components		96 determinations	48 determinations
1.	Microelisa stripplate	12well×8strips	12well×4strips
2.	Standard	0.5ml×6bottle	0.5ml×6bottle
3.	Concentrated biotinylated antigen	18μl×1 bottle	9μl×1 bottle
4.	Concentrated avidin-HRP	6μl×1 bottle	3μl×1 bottle
5.	Biotinylated antigen diluent	6ml×1 bottle	3ml×1 bottle
6.	Avidin – HRP diluent	6ml×1 bottle	3ml×1 bottle
7.	Chromogen Solution A	6ml×1 bottle	3ml×1 bottle
8.	Chromogen Solution B	6ml×1 bottle	3ml×1 bottle
9.	Stop solution	6ml×1 bottle	3ml×1 bottle
10.	25X Wash solution	20ml×1 bottle	20ml×1 bottle
11.	Closure plate membrane	2	2
12.	User manual	1	1
13.	Sealed bags	1	1

Note: Standard (A → F) concentration was followed by: 0ng/ml,10ng/ml ,20ng/ml ,

40ng/ml ,80ng/ml ,160ng/ml.

MATERIALS REQUIRED BUT NOT PROVIDED

1. 37℃thermostat.

2.Standard ELISA.

3. Precision pipette and disposable tip

4. Double distilled water or ultrapure water

5. Clean test tube or Eppendof tube

6. Absorbent paper

7. Automatic plate washer or 8-channel pipette

8. ELISA data processing software, ELISACalc recommended

9. 500ml beaker and suitable measuring cylinder

Precautions

1.Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.

2.It is highly recommended to use the remaining reagents within 1 month before the deadline. For the expiration date, please refer to the label on the kit box. All components are stable before this expiration date.Do not use kit components beyond their expiration date.

3.Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.

4.Use only deionized or distilled water to dilute reagents.

5.Each steps add sample, should use sampler, and often proofread the accuracy to avoid the test error. Use fresh disposable pipette tips for each transfer to avoid contamination.

6.Test should strict accordance with the instructions of the operation, the test results must be determined by the microplate reader.

7.Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.

8.Do not mix acid and sodium hypochlorite solutions.

9.Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from Rat blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

10.All samples should be disposed of in a manner that will inactivate viruses.

11.Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand

for a minimum of 30 minutes to inactivate the viruses before disposal.

12.Substrate Solution is easily contaminated. If bluish prior to use, do not use. Substrate B is sensitive to light and avoid prolonged exposure to light.

Sample collection and storages

1.**Serum:** Allow the serum to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RPM) for 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

2.**Blood plasma**: In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as anti coagulation. Add EDTA or sodium citrate and mix them for 10-20 minutes. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

3.**Urine**: Collect by sterile tube. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow the procedures above-mentioned.

4.**Cell culture supernatant**: Collect by sterile tubes when examining secrete components. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (PH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

5.**Tissue sample**: Incise sample and weigh up. Add a certain amount of PBS (PH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8°C. Add a certain amount of PBS (PH 7.4) and then homogenize the sample thoroughly by hand or homogenizer. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

Note: 1.Samples to be used within 5 days may be stored at 2-8℃, otherwise samples must be stored at -20℃ (≤1month) or -80℃ (≤2 months) to avoid loss of bioactivity and avoid contamination.

2.Sample hemolysis will influence the result, so the samples should be centrifuged adequately and no hemolysis or granule was allowed.

3.When performing the assay, bring samples to room temperature.

Samples containing NaN3 can't be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).

4.After collecting the sample, extraction should be immediately carried out in accordance with related documents. After extraction, experiment should be conducted immediately as well. Otherwise, keep the sample at -20℃. Avoid repeated freeze-thaw cycles.

Reagent preparation

a.The sample can be directly add in this kit. If the concentration is too high, it's possible to be diluted according to suitable proportion (2-5X dilution is recommended). The result shall be multiplied by dilution times during the calculation.

b.The wash buffer is 25X concentrated solution. Before using, pour all wash buffer into 500ml or 1L beaker and use double distilled water for constant volume of 500ml, i.e.working solution.

c.Dilution of biotinylated antigen: use the mini centrifuge for centrifugation and draw out 1ml biotinylated antigen diluent to concentrated biotinylated antigen and make vortex mixing for 15 seconds and then pour all solution to the diluents bottle to get the working solution of biotinylated antigen. (before dilution to avoid repeated freeze-thaw cycles.)

d.Dilution of avidin-HRP: use the mini centrifuge for centrifugation and draw out 1ml avidin-HRP diluent to concentrated avidin-HRP and make vortex mixing for 15 seconds and then pour all solution to the diluents bottle to get the working solution of avidin-HRP. (before dilution to avoid repeated freeze-thaw cycles.)

Washed plate method

1.Hand-washed plate method: get rid of the liquid within the ELISA plate; in the experimental bench paved a few layers of absorbent paper, pat hard the ELISA plate several times downward; the diluted washing solution at least 0.35ml inject into the well, soaking 1-2 minutes. Repeat this process several times as needed.

2. Automatic plate washing: If you have automatic washing machine, Should be skilled use, and then used in the formal experiment process.

Assay procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.

2.Do not add the blank well, only add Chromogen Solution A, B and stop solution for zero setting.

3.Standard well: add 50μl Standard dilution each well and add 50μl Standard/sample dilution in the zero well, and then add 50μl working solution of biotinylated antigen.

4.Sample well: add 50μl sample (recommend to add the sample directly and use the sample dilution for 2-5X dilute if the concentration is high) and then add 50μl working solution of biotinylated antigen.

5.Cover it with seal plate membrane, gently shake and mix for 60 minutes at 37 ° C incubation.

6.Preparation of washing solution: Dilute the washing concentration (25X) with distilled or deionized water for later use.

7.First washing: carefully remove the sealing film, drain the liquid, dried up, each well filled with washing solution, put it aside for 1 min then drain the liquid, so repeat 5 times, pat dry. (Automatic washing: Each wells inject into the wash solution 350μL, soak 1min, wash plate 5 times.)

8.Add the 50μl avidin-HRP in Standard well and sample well and cover it with seal plate membrane, gently shake and mix for 60 minutes at 37 ° C incubation.

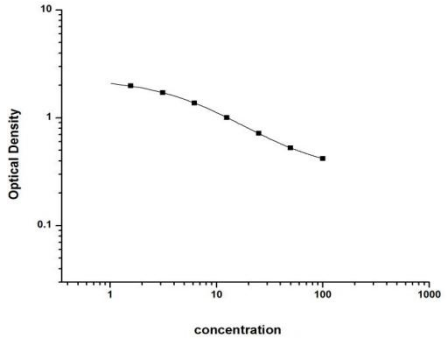
9.Second washing: carefully remove the sealing film, drain the liquid, dried up, each well filled with washing solution, put it aside for 1 min then drain the liquid, so repeat 5 times, pat dry. (Automatic washing: Each wells inject into the wash solution 350μL, soak 1min, wash plate 5 times.)

10.Color developing: add 50μl Chromogen Solution A to each well and then add 50μl Chromogen Solution B to each well as well. Shake gently to mix up. Incubate for 15 minutes at 37℃,away from light for color developing.

11.Stop: Add 50μl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment). If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

12.Assay: Take blank well as zero, measure the absorbance (OD) of each well one by one under 450nm wavelength, which should be carried out within the 15 minutes after having added the stop solution.

13.According to standards' concentrations and the corresponding OD values, to calculate the four parameters equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample. Also can use related application software.



1.Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.

The Above standard curve only for demonstration purposes

2.Intra-assay CV(%) is less than 10% and Inter-assay CV(%) is less than 15%.

Summary of operating procedures

Prepare reagents, samples and standards

Add prepared samples and standards,and biotinylated antigen and 37℃ react for 60 minutes

Wash the plate 5 times, add avidin-HRP and 37℃ react for 60 minutes

Wash the plate 5 times and add chromogen solution A, B, 37°C developing color for 15 minutes

Add the stop solution

Read the OD value within 15 minutes

Calculate

FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS! PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

Any further information contact bio@coonkoon.com