IMMUNOPORT

SKU IP700004

FELINE CORTISOL (COR) ELISA KIT

Research Use Only
For *in vitro* applications - not for consumption

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VERSION 1.3

INTENDED USE

The kit is competitive enzymatic immunoassay for quantitative determination of Feline Cortisol(COR) concentrations in serum, blood plasma, saliva, urine, tissue homogenates, or related biological samples.

KIT CONTENTS & STORAGE

Microelisa Plate [12 X 8 Strips]
Standards A to F [0, 5, 10, 20, 40, 80ng/mL - 0.5ml each]
Concentrated biotinylated antigen [18µL]
Concentrated avidin-HRP [6µL]
Biotinylated antigen diluent [6ml]
Avidin – HRP diluent [6ml]
Chromogen Solution A and B [6ml each]
25X Wash solution [20ml]
Stop Solution [6ml]
Plate Sealing Membrane [2pcs]
User manual [1pc]
Zipper bag [1pc]

METERIALS REQUIRED BUT NOT SUPPLIED

ELISA Reader, 37 °C Thermostat, micropipettes and tips, Eppendorf tubes, absorbent paper, autowasher, deionized or distilled water.

SUMMARY

This kit is based on the principle of competitive inhibition enzyme linked immunosorbent assay. In brief, samples (and standards) containing the analyte are added to the micro-titre wells that are pre-coated with a protein which captures the analyte. Subsequently HRP-conjugated reagent(s) is(are) added to form an immune complex. This is followed by incubation and washing, removal of unbound enzyme, and sequential addition of chromogen which turns blue and finally changes into yellow color under the effect of acid. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of analyte in the sample.

CHARACTERISTICS

Sample Type: serum, plasma, cell culture supernatants, urine, saliva and biological fluids.

Detection Range: 2.5ng/mL -80ng/mL
Minimum Detection Dose: 1.0ng/mL
Intra-Assay Precision: CV<9%
Inter Assay Precision: CV<11%

Cross Reactivity: None observed with analogues
Validity: 1 year from the date on manufacture
Storage: 2-8°C

Once opened unused wells should be put into the sealed bag with a desiccant pack and may be sorted for up to 1 month at 2-8 °C.

SAMPLE COLLECTION

Tissue homogenates - Incise the sample and weigh up. Add a certain amount of PBS (PH 7.4), 9ml PBS in 1mg tissue sample. Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8℃. 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.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at 1000×g. 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 - Add EDTA or heparin as an anticoagulant in the sample and mix it for 10-20 minutes. Then Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection. Collect the supernatants carefully and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Note: If sediments occur during storage, centrifugation should be performed again**.** 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.

REAGENT PREPARATION

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

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 making the working solution.

c.Dilution of biotinylated antigen: draw out 1ml biotinylated antigen diluent to concentrated biotinylated antigen and vortex for 15 seconds and then pour the solution to the diluents bottle to get the working solution of biotinylated antigen. (avoid repeated freeze-thaw cycles.)

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

PROCEDURE

1. Bring all reagents to room temperature before use. It is recommended that all Standards and Samples be performed in duplicate. Do not add anything to the blank well, only Chromogen Solution A, B and stop solution need to be added to it.

2. Standard wells: Add 50µl Standards to each respective well and add 50µl Standard/Sample diluent in the zero well, and then add 50µl working solution of biotinylated antigen.

3. Sample wells: Add 50µl sample and then add 50µl working solution of biotinylated antigen.

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

5. First Washing: Use Diluted (1X) Wash solution. Carefully remove the sealing film, drain the liquid by patting on absorbent paper. Then fill each well with washing solution, hold for 1 min and then drain the liquid and pat on absorbent paper. Repeat 5 times.

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

7. Second Washing: Do the same as in step 5.

8. Color Development: 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°C, away from light for color development.

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

11. 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. Calculate the concentration of the corresponding sample.

CALCULATION

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between analyte concentration in the sample and the assay signal intensity.

Average the duplicate readings for each standard, control, and samples. Create a standard curve with the log of analyte concentration on the y-axis and absorbance on the x-axis. Draw a best fit curve through the points and it can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

We have compiled a list of most common errors and resolutions on our technical support webpage www.immunoport.com/scientific-support. Still if you have any concerns please feel free to write to us at info@immunoport.com

PRECAUTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- 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 blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Add sodium hypochlorite to a final concentration of 1.0% in the liquid waste generated. The waste should be allowed to stand for a minimum of 30 minutes to inactivate any viruses before disposal.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

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