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User's Manual

Canine adrencocorticotropic hormone ELISA Kit

REF **DEIA-CL020**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This kit is used for the quantitative determination the concentrations of Canine Adrenocorticotropic Hormone (ACTH) in canine serum, plasma, cell culture supernatants, and other biological fluids.

Important notes (read before start)

1. The kit should be balanced 15-30 minutes in the room temperature after taken out from the refrigeration environment. The microplate should be stored in **sealed bag and valid for 30 days after open**.
2. Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from contamination.
3. Washing buffer may have crystallized precipitates, heating the solution can dissolve the precipitates.
4. Add samples as soon as possible. Adding solutions by multi-channel pipettes is highly recommended.
5. Dilute the samples before the assay by the same buffer which has been used to extract the samples, if the concentration of original samples is higher than the assay range.
6. Duplicated loading of all standards and samples is highly recommended.
7. If OD450 in both standard and samples are very low, the incubation time for each step can be extended.
8. The substrates are light sensitive; avoid any unnecessary exposure of the substrate under the light. Avoid any contact of the substrates with metal which prevents color development.
9. Due to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g. antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins/molecules may not be recognized by this kit.
10. Influenced by the factors including cell viability, cell numbers, samples stored for long time, or samples from cell culture supernatant without concentrated, these samples may not be detected by this kit.
11. Samples may be stored at 2-8°C if test happens within one week after collection. Otherwise, samples must be stored at -20°C for less than two months or -80°C for less than six months.
12. Customer is responsible for the sample consumed during the assay. Customer should calculate the possible amount of the samples used in the entire assay and reserve sufficient samples in case of any testing issue.
13. Do not mix or interchange reagents between different lots.
14. Do not use reagents beyond the expiration date of the kit.

Principles of Testing

The kit assays canine ACTH in the samples by the method of double-antibody sandwich ELISA. The solid-phase antibody is prepared by purified canine ACTH antibody which is pre-coated onto a microplate. After loading of samples, the ACTH in the standards or samples bind with the coated antibody. Following by adding biotinylated-ACTH antibody, the HRP-Streptavidin enzyme conjugate has been added into wells. After washing to remove any unbound antibody -enzyme reagent, the tetramethylbenzidine (TMB) substrate is added. TMB substrate produces blue color when catalyzing by the HRP enzyme. After reaching the desired color intensity, the reaction is terminated by addition of an acidic S top solution which changes the solution color from blue to yellow. Determining thee absorbance (OD Value) at wave length 450 nm by the microwell reader, and the concentration of ACTH can be calculated according to standard curve.

Reagents And Materials Provided

1	Microplate (pre-coated)	96 wells	5	Enzyme Conjugate	12 ml × 1 bottle
2	10 x Sample Buffer	12 ml x 1 bottle	6	20 x Wash Solution	50 ml × 1 bottle
3	Standards	160 ng × 2 bottles	7	Substrate Solution	12 ml × 1 bottle
4	Biotinylated Antibody	6 ml x 1 bottle	8	Stop Solution	12 ml × 1 bottle

Storage

Storage: 2-8°C.

Shelf time: Six months.

Specimen Collection And Preparation

1. Extract samples as soon as possible after specimen collection according to the relevant literatures. Predict the concentration before assaying. If values are not within the range of the standard curve, customer needs to determine the optimal sample dilutions for their particular experiments. Samples subject to be determined as soon as possible after the extraction. Otherwise, specimen needs to be stored in -20 to -80°C and avoid repeated freeze-thaw cycles.

Note: Can't detect the sample which contains NaN3, because NaN3 inhibits HRP activity.

2. Serum, plasma, cell culture supernatants, urine samples do not need extraction.
3. Washing & Sample solutions: Diluting 20-fold washing solution to 1X with distilled water (example: 1 ml concentrated washing liquid needs 19 ml distilled water). Diluting 10-fold sample solution to 1X with distilled water (example: 1 ml concentrated sample solution needs 9 ml distilled water).
4. Standard solution preparation: Add 2 ml distilled water into one standard bottle to make the standard solution as 80 ng/ml. Prepare 8 clean 1.5 ml tubes. Then add 900 µl of 1 X Sample Buffer into the first tube, add 500 µl of 1 X Sample Buffer into the second to the eighth tube separately. Add 100 µl of standard solution (80 ng/ml) to the first tube. After mixed with a pipette, transfer 500 µl of solution to the second tube. Then repeat the mixing and transfer from third to seventh tubes. Leave the eighth tube as blank. The finally standard concentration in each tube should be shown as the table below (pg/ml):

1	2	3	4	5	6	7	8
8000	4000	2000	1000	500	250	125	0

Assay Procedure

1. Sample loading: Add 100 µl of standards (prepared above) or sample to each well, **don't touch the well wall** and mix **gently**.
2. Incubation: Incubate the plate at 37°C for 40 min. A plate layout is provided to record standards and samples assayed.
3. Wash: Discard the solution by aspirating or decanting, then add 200-300 µl washing buffer to each well. Complete removal of liquid at each step is essential to good performance. Repeating the process 4-6 times. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels each time.

4. Add antibody: Add 50 µl of distilled water and 50 µl Biotinylated Antibody into each well (**except blank well**) and incubation at 37°C for 20 min.
5. Repeat wash as in step 3.
6. Add enzyme: Add 100 µl of Enzyme Conjugate to each well. Incubating the plate at 37°C for 10 min.
7. Repeat wash as in step 3.
8. Develop: Add 100 µl Substrate Solution to each well, and incubate at 37°C for 15 min. Protect from light.
9. Add 100 µl of Stop Solution to each well and mix well.
10. Read absorbance at 450 nm within 30 min after adding Stop Solution.

Calculation

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Plot the optical density for the standards versus the concentration of the standards and draw the best curve. To determine the ACTH concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding ACTH concentration. If the samples have been diluted, the concentration read from the standard curve should be multiplied by the dilution factor.

Performance Characteristics

1. Sensitivity: less than 60 pg/ml.
2. Specificity: simultaneous detection of recombinant or natural canine ACTH without cross-reaction with other cytokines.
3. Repeatability: both inter-assay and intra-assay were less than 10 %.

Precautions

1. The Elisa Kit is for research use only. Not for diagnostic or other purposes.
2. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
3. The substrates are toxic; Avoid direct contact with hands. Dispose off properly.
4. Do not eat, drink, smoke or apply cosmetics where kit reagents are used. Do not pipette by mouth.