Canine Interleukin 4 (IL-4) ELISA Kit

Canine Interleukin 4 ELISA Kit
Catalog Number: ECI0014 (96 Tests)

This kit is to detect Canine Interleukin 4 . Compatible sample types: serum, plasma, cell culture supernatants, tissue homogenate, body fluid.
Store all reagents at 2-8°C.

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS. READ THROUGH ALL PROCEDURES BEFORE USE.

ASSAY PRINCIPLE

The Interleukin 4 ELISA is a quantitative competitive immunoassay. The plate provided is coated with Interleukin 4 specific antibody. Standards and experimental samples are co-incubated in wells along with Interleukin 4 -HRP conjugate. Interleukin 4 in standards and samples competes with the Interleukin 4 -HRP conjugate for binding to the plate-bound antibody. Higher levels of Interleukin 4 from standards or samples lead to decreased Interleukin 4-HRP conjugate binding and thus reduced signal. Captured Interleukin 4-HRP is quantitatively detected by incubation with HRP substrates (substrates A and B). Binding of the Interleukin 4-HRP is visualized by production of colorimetric reaction products that can be quantitatively measured by EMR absorbance at 450nm.

MATERIALS

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

1	Wellplate	96 strip wells	
2	Standard A	0 pg/ml	0.5 ml
3	Standard B	50pg/ml	0.5 ml
4	Standard C	100pg/ml	0.5 ml
5	Standard D	250pg/ml	0.5 ml
6	Standard E	500pg/ml	0.5 ml
7	Standard F	1000pg/ml	0.5 ml
8	Substrate A	1 vial	6.0 ml
9	Substrate B	1 vial	6.0 ml
10	Stop Solution	1 vial	6.0 ml
11	Enzyme Solution	1 vial	6.0 ml
12	Balance Solution	1 vial	1 ml
13	Wash Solution (25×)	1 vial	50 ml

GENERAL CONSIDERATIONS

1. Samples should be handled following standard practices to minimize degradation or denaturation. Avoid multiple freeze-thaw cycles and high temperatures. For long term storage, maintain samples at temperatures that minimize degradation or denaturation (-20C is recommended).
2. Without prior knowledge of the analyte concentration, determining the amount of sample required for robust detection is difficult. Therefore, we recommend that enough sample be collected to allow for multiple dilutions to be assayed.
3. Experimental variation between wells is to be expected. We recommend assays be performed using AT LEAST two wells for every sample or standard. Readings of duplicate wells should be averaged.
4. Two blank wells containing only PBS or DI H2O should be tested to determine background.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature before use.
2. Bring Plate to room temperature before opening. Remove the desired number of well strips and immediately reseal and store at 2-8°C.
3. Dilute 40 mL of Wash Solution concentrate (25×) with 960 mL of deionized or distilled water. If crystals have formed in the concentrate warm to room temperature and mix to dissolve.

SAMPLE PREPARATION

1. Using serum or plasma directly without any dilution (neat) will often give more accurate results than any other sample type.
2. If samples are not serum or plasma, or have been diluted, 10µL of Balance Solution is required to be added into 100µL experimental samples. Balance solution is recommended for diluted serum or plasma samples.

ASSAY PROCEDURE

1. Add 100µL of sample or standards to the appropriate number of wells in the supplied wellplate. Note that wells have been pre-blocked and no additional blocking steps are required. Add 100µL of PBS (pH 7.0-7.2) or water to the blank well.
2. Add 50µL of Enzyme Solution to each well (**but NOT blank well**) in the supplied Plate and mix well.
3. Cover and incubate 1 hour at 37^{0}C in a humid chamber.
4. Wash each well 5 times with 300-400µL 1X Wash Solution per well. After the last wash invert the plate and blot dry by tapping on absorbent paper. Note: Hold the sides of the plate frame firmly when washing to assure that all strips remain **securely** in the frame. Complete removal of liquid at each step is essential for best performance of the assay.
5. Add 50µL Substrate A to each well followed by addition of 50µL Substrate B. Cover and incubate 10-15 minutes at room temperature. **Substrate is light sensitive**. Keep out of direct sunlight or cover with foil.
6. Add 50µL of Stop Solution to each well and mix well.
7. Immediately read the optical density (O.D.) at 450 nm.
8. Subtract the mean blank value from each sample or standard value and calculate the mean for duplicate (or greater) wells.
9. Construct the standard curve using graph paper or statistical software.

Note: **Blank well must NOT have Enzyme Solution.**

SENSITIVITY AND SPECIFICITY

1. The sensitivity of this assay should be approximately 1.0pg/ml .
2. This assay has high sensitivity and excellent specificity for the detection of Interleukin 4 . No significant cross-reactivity or interference between Interleukin 4 and any homologous proteins assayed has been observed. Cross-reactivity with other species has not been specifically determined.

ADDITIONAL SUGGESTIONS

1. When not in use, kit components should be refrigerated (4C for short-term use, or -20C for long-term storage). All reagents should be warmed to room temperature before use.
2. Samples should be collected in pyrogen/endotoxin-free tubes.
3. When possible, avoid use of hemolyzed or lipemic sera. Centrifuge or filter samples if particulate matter is present.
4. It is recommended that all standards, controls, and samples be run at least in duplicate.
5. To insure equal incubation times, maintain a consistent order of addition from well-to-well when pipetting reagents.
6. Cover or cap all reagents when not in use.
7. Do not mix or interchange different reagent lots from various kit lots.

8. Do not use reagents after the kit expiration date.
9. Determine absorbance within 2 hours of assay completion.
10. The provided standards should be run with every assay.
11. Substrate B is light sensitive. Avoid prolonged exposure to light. Substrate B will discolor metals, so contact with metals should be avoided.
12. Incomplete washing will adversely affect the test outcome. All washing must be performed with the provided Wash Solution.
13. Washing can be performed using a squirt bottle and filling all wells to the top.
14. Standards and samples must be assayed at the same time. Do not mix reagents from different lots. Again, it is recommended that assays be performed at least in duplicate.
15. This kit might be not suitable for some peptides or recombinant proteins. If samples were peptides or recombinant proteins, please consult with our technical team before use.

SAFETY NOTES

1. This kit contains 3, 3', 5, 5'-Tetramethylbenzidine (TMB) in Substrate B. TMB, present at levels greater than or equal to 0.1% is NOT identified as a carcinogen or potential carcinogen by OSHA. TMB may cause irritation to skin and eyes. Please wear appropriate personal protective equipment, including gloves, safety glasses, and lab coats when handling.
2. The Stop Solution provided in the kit is an acidic solution. Please wear appropriate personal protective equipment (gloves, safety glasses, lab coat) when handling this and all kit components.
3. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
4. All waste must be disposed of in accordance with all applicable local, state, and federal regulations.

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Calculation of competitive ELISA results

1. Original data (example)

OD	Concentration of standards
2.1515	0
1.4933	50
1.1893	100
0.8247	250
0.5132	500
0.2124	1000

2. Data processing

   X: O.D. of other non-zero standards divided by that of Standard 0, then multiplied by 100;

   Y: log(base 10 or other) of (concentration of standards)

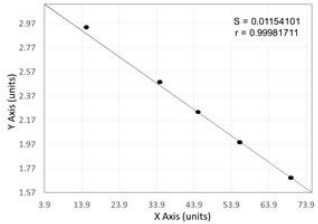
X	Y
100	0
69.407	1.698970004100
55.277	2
38.331	2.397940009
23.853	2.698970004
9.872	3

3. The standard curve is generated using some curve software (standard 0 is not used).
4. The formula:

   Linear Fit: y = a+bx
   Coefficient Data: 0.998
   a = 3.22E+00
   b = -2.19E-02

5. Calculation of results

   1) To process the sample O.D.: O.D. of samples divided by that of Standard 0, then multiplied by 100;
   2) To get Y value using the above formulation
   3) To get the concentration of samples: 10 to the power of Y value.



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