Fast Canine IL-4 ELISA kit

For the quantitation of canine IL-4 concentrations in cell culture supernatants, serum, and plasma.

INTRODUCTION

Interleukin-4 (IL-4) is a secreted protein which belongs to the IL-4 / IL-13 family. IL-4 enhances both secretion and cell surface expression of IgE and IgG1. It regulates the expression of the low affinity Fc receptor for IgE (CD23) on both lymphocytes and monocytes. IL-4 is essential for the switching of B cells to IgE antibody production and for the maturation of T helper (Th) cells toward the Th2 phenotype. It participates in at least several B-cell activation processes.

The Fast Canine IL-4 ELISA is a solid phase ELISA designed to measure canine IL-4 levels in cell culture supernatants, serum, and plasma. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 2 hours, not 5-6 hours (Fig. 1). The detection range is from 78 to 5000pg/mL. The levels of canine IL-4 samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural canine IL-4 protein.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (See Fig. 1). A monoclonal antibody specific for canine IL-4 was pre-coated onto a microplate. Standards or samples and detection antibody are pipetted into the wells, then, concurrently incubated to form a sandwich complex in one-step. Simply aspirate each well without wash, directly add Streptavidin-HRP into the complex. Following a wash, an ultra-sensitive TMB substrate solution is added to the wells for color develops. The color intensity is in proportion to the amount of IL-4 bound in the initial step. The intensity of the color is measured by plate read at 450 nm.
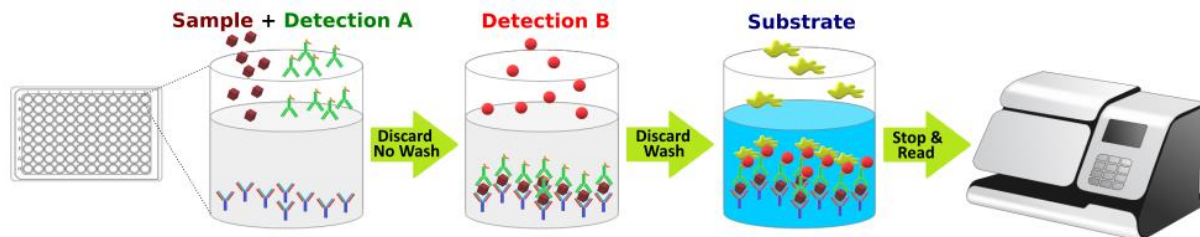

Fig.1 Simple ELISA procedure.

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Canine IL-4 Microplate	TBS33004A	96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for Canine IL-4.	The unused wells can be stored in the sealed foil pouch containing the desiccant pack for up to 1 month at 2-8 ℃.
Canine IL-4 Standard	TBS33004B	60 μL of Recombinant Canine IL-4 protein (100ng/mL).	Aliquot and store at -20 ℃ for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS33004C	2.1 mL of Biotinylated Canine IL-4 antibody.	May be stored for up to 3 months at 2-8 ℃.
Detection B	TBS33004D	12 mL of Streptavidin-HRP	
Assay Diluent	TBS33004E	12 mL of a buffered protein base with preservatives.	
10x Wash Buffer	TBS3000W	12 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS
Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION
Bring all reagents to room temperature before use.

Wash Buffer: Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

Canine IL-4 Standard Preparation:

1. Label test tubes as #1 through #8. Pipet 475 µL of 1x Assay Diluent into tube #1, and 250 µL into tubes #2 to #8 **as diagram below.**
2. Add 25 µL of the Canine IL-4 Standard stock solution (100ng/mL) by dilution of 20X to tube #1 and mix.
3. Make 2x serial dilutions of the standard using the 5000pg/mL standard solution from tube #2 through #7 with sequential transfer of 250 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 5000, 2500, 1250, 625, 312.5, 156 and 78.1 pg/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Canine IL-4 standard preparation

Addition: 25µL   250µL   250µL   250µL   250µL   250µL   250µL
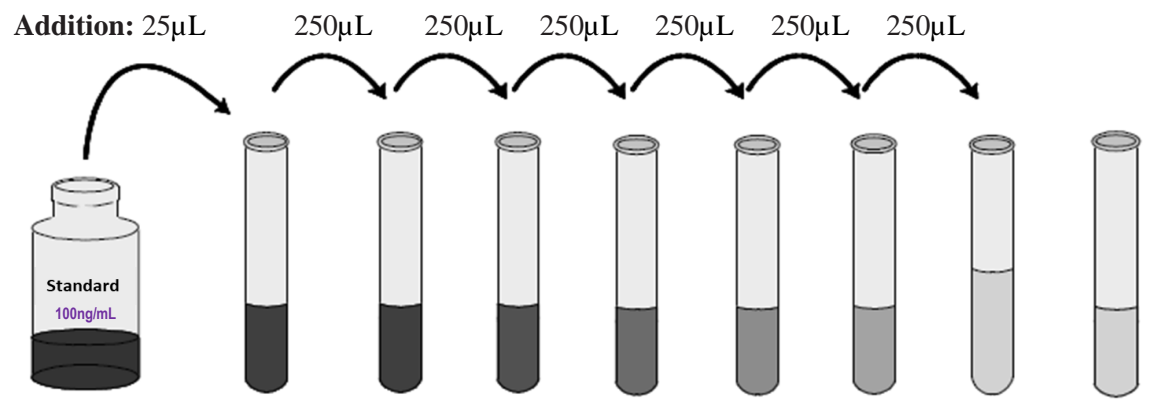
Standard	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	475	250	250	250	250	250	250	250
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol (µL)	25	250	250	250	250	250	250	0
Final Conc (pg/ml)	5000	2500	1250	625	312.5	156.25	78.125	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use (*It is very important for good performance*). It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours.**
3. Aspirate each well (*no wash*). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B** to each well. Incubate at **RT for 20 min.**
5. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes

blue**.** If the color is light, the incubation time can be longer.

7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).

8. Determine the optical density of each well within 5 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

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

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the canine IL-4 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve (R^2=1) is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

SENSITIVITY

The minimum detectable dose (MOD) of canine IL-4 is typically 50 pg/ml.

The Intra-CV is < 10%, the Inter-CV is < 10%.

SPECIFICITY

This assay recognizes natural and recombinant canine IL-4.
No-cross reactivity:
Canine IL-2; canine IL-6; bovine IL-4; cotton rat IL-4; equine IL-4; feline IL-4; human IL-4; mouse IL-4; porcine IL-4; rabbit IL-4; rat IL-4; rhesus macaque IL-4.

RELATIVE PRODUCTS

Canine IL-1ß ELISA (TBS33001)
Canine IL-2 ELISA (TBS33002)
Canine IL-6 ELISA (TBS33006)
Canine IL-7 ELISA (TBS33007)
Canine IL-8 ELISA (TBS33008)
Canine IL-12/IL23 ELISA (TBS33012)
Caine GM-CSF ELISA (TBS33014)
Canine IL-17A ELISA (TBS33017)
Canine IL-18 ELISA (TBS33018)
Canine IL-20 ELISA (TBS33020)
Canine IFN-gamma ELISA (TBS33026)
Canine TGF- ß1 ELISA (TBS33030)
Canine Insulin ELISA (TBS33034)
Canine MIP-1α ELISA (TBS33035)
Canine TNF-α ELISA (TBS33040)

Fig.3 Canine IL-4 Standard Curve
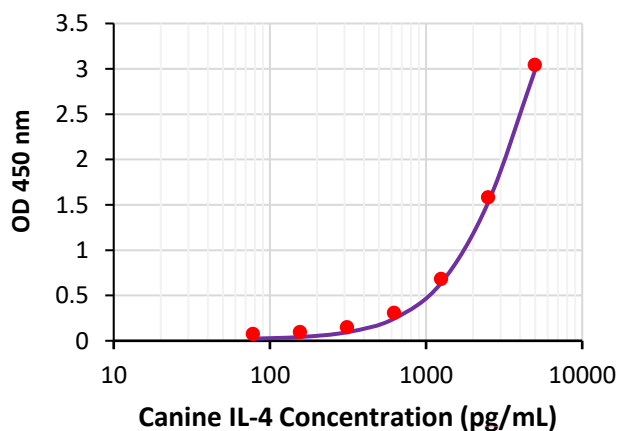
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