Canine IL-2 ELISA kit Catalog Number: TBS33002

INTRODUCTION

Interleukin 2 (IL-2) is a monomeric glycoprotein and a type I four-α-helical bundle cytokine. It is primarily produced by activated CD4+ and CD8+ T cells, as well as dendritic cells. IL-2 is essential for the proliferation and differentiation of T cells, B cells, and natural killer (NK) cells. It plays a role in both pro-inflammatory and immune-regulatory responses. IL-2 enhances the cytolytic activity of NK cells and is crucial for immune response regulation. It has been used in cancer immunotherapy, particularly for metastatic melanoma and renal cell carcinoma.

The Canine IL-2 Fast ELISA is a solid phase ELISA designed to measure canine IL-2 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 62 to 4000 pg/mL. The levels of canine IL-2 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-2 protein.

Alternative name: Aldesleukin; IL2; IL-2; IL-2lymphokine; interleukin 2; interleukin-2; involved in regulation of T-cell clonal expansion; Proleukin; T cell growth factor; T-cell growth factor; TCGF

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (See Fig. 1). A monoclonal antibody specific for canine IL-2 was pre-coated onto a microplate. Standards or samples and a biotin-conjugated detection antibody are pipetted into the wells, then, concurrently incubated to form a sandwich complex in one-step. Simply aspirate each well without washing, directly add Streptavidin-HRP into the complex. Following a wash, an ultra-sensitive TMB substrate solution is added to the wells for color development. The color intensity is in proportion to the amount of IL-2 bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

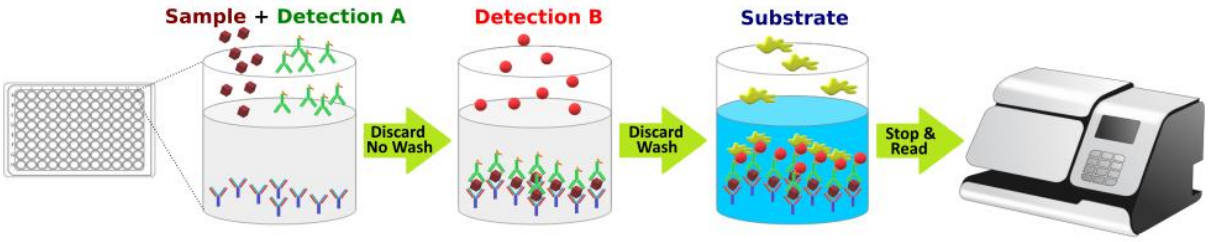


Fig.1 Simple ELISA procedure.

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Canine IL-2 Microplate	TBS33002A	96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for Canine IL-2.	The unused wells can be stored in the sealed foil pouch containing the desiccant pack for up to 1 month at 2-8 °C.
Canine IL-2 Standard	TBS33002B	30 µL of Recombinant Canine IL-2 protein (200 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS33002C	220 µL of Biotinylated Canine IL-2 antibody.	May be stored for up to 3 months at 2-8°C.
Detection B	TBS33002D	300 µL of Streptavidin-HRP	
Assay Diluent	TBS33002E	15 mL of a buffered protein base with preservatives.	
10x WashBuffer	TBS3000W	15 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 °C. 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, eyes, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all the reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved*).

Detection A working solution preparation: Add 210 µL of Detection A Stock (Biotin-canine IL-2 antibody) to 1890 µL Assay Diluent to prepare Detrection A working solution. Add 20 µL to each well.

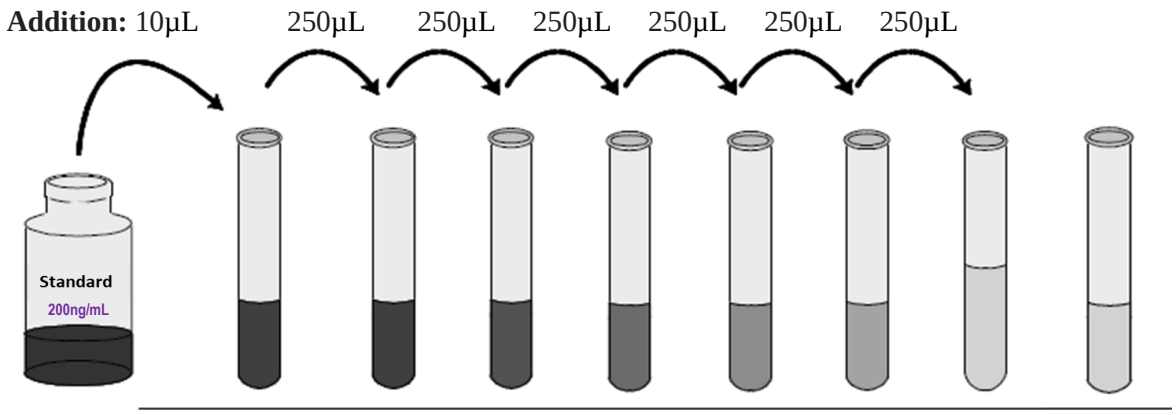
Detection B working solution preparation: Add 240 µL of Detection B streptavidin-HRP to 12 mL Assay Diluent to prepare Detrection B working solution. Add 100 µL to each well.

Canine IL-2 Standard Preparation:

Label test tubes as #1 through #8. Pipet 490 µL of 1x Assay Diluent into tube #1, and 250 µL into tubes #2 to #8 **as diagram below.**

1. Add 10 µL of the canine IL-2 Standard stock solution (200 ng/mL) by dilution of 50X to tube #1 and mix.
2. Make 2x serial dilutions of the standard using the 4000 pg/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 4000, 2000, 1000, 500, 250, 125 and 62.5 pg/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Canine IL-2 standard preparation



Standard	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	490	250	250	250	250	250	250	250
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol (µL)	10	250	250	250	250	250	250	0
Final Conc (pg/mL)	4000	2000	1000	500	250	125	62.5	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-2 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. Fig. 3 is an example of typical Data.

SENSITIVITY

The minimum detectable dose (MDD) of canine IL-2 is typically 62 pg/mL.
The Intra-CV is < 10%, the Inter-CV is < 10%.

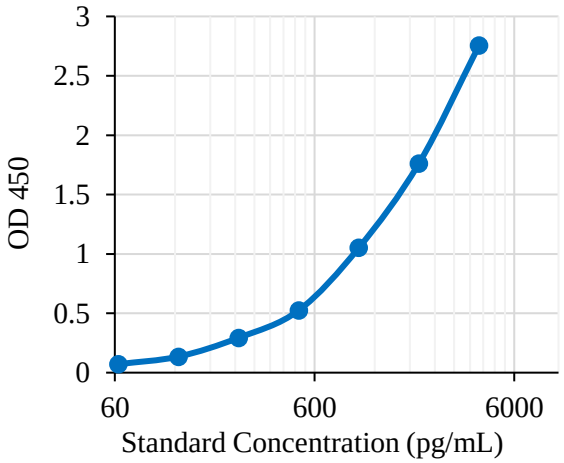
SPECIFICITY

This assay recognizes natural and recombinant canine IL-2.

RELATIVE PRODUCTS

Canine IL-1β ELISA (TBS33001)
Canine IL-4 ELISA (TBS33004)
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-2 Standard Curve



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