Tribioscience

Canine (Dog) IL-8 /CXCL8 Fast ELISA (Catalog Number: TBS33008)

For quantitation of canine IL-8 concentrations in cell culture supernatants, serum, and plasma

INTRODUCTION

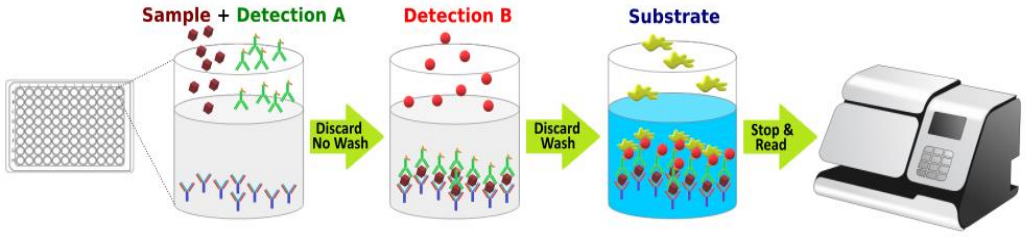
Interleukin 8 (IL-8, also as CXCL8) is a chemokine with a defining CXC amino acid motif, and has tumorigenic and proangiogenic properties. This chemokine is secreted by a variety of cell types including monocyte/macrophages, T cells, neutrophils, fibroblasts, endothelial cells, and various tumor cell lines in response to inflammatory stimuli (IL1, TNF, LPS, etc). This chemokine has crucial roles in various pathological conditions such as chronic inflammation and cancer. IL-8 has been associated with tumor angiogenesis, metastasis, and poor prognosis in breast cancer.

The Canine (Dog) IL-8 Fast ELISA is a solid phase ELISA designed to measure canine/dog IL-8 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 1-2 hours, with no need for 4-5 hours (Fig. 1). The detection range is from 8 to 2000 pg/mL. The levels of canine IL-8 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-8 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-8 was pre-coated onto a microplate. Standards or samples and Detection Antibody are pipetted into the wells, and concurrently incubated for 45min. Then, aspirate each well, no wash, directly add Streptavidin-HRP, incubate the complex. Following a wash to remove any unbound antibody and samples, 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-8 bound in the initial step. The intensity of the color is measured by plate read at 450 nm.µ

Fig.1: Assay Procedures



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Canine IL-8 Microplate	TBS33008A	96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for canine IL-8.	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-8 Standard	TBS33008B	15 µL of Recombinant canine IL-8 protein (100 ng/mL).	Aliquot and store at -20 ℃ for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS33008C	2.2 ml of canine IL-8 antibody.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS33008D	120 µL of Streptavidin-HRP (100x)	
Assay Diluent	TBS33008E	25 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.

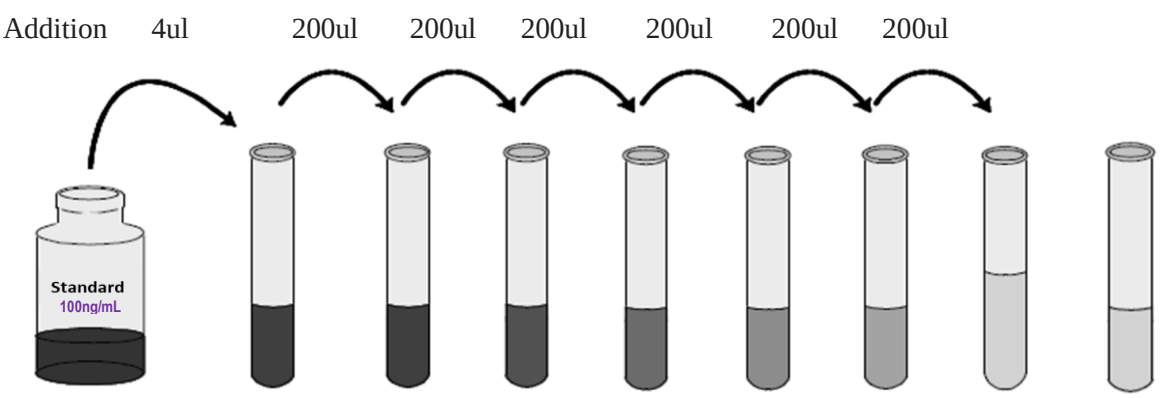
Wash Buffer: Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

Detection B working solution preparation: Add 100 µL of Detection B HRP conjugated secondary antibody to 10 mL Assay Diluent to prepare Detrection B working solution.

Canine IL-8 Standard Preparation:

1. Label test tubes as #1 through #8. Pipet 396 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 **as diagram below.**
2. Add 4 µL of the canine IL-8 Standard stock solution (100ng/mL) by dilution of 100 times to tube #1 (1000pg/mL) and mix. Then make 2 x serial dilutions of the standard using the 1000pg/mL standard solution in tube#1 from tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 1000, 500, 250, 125, 62.5, 31.3 and 15.6 pg/mL. Tube# 8 is Standard 0.

Fig.2: Diagram for canine IL-8 standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	**396**	200	200	200	200	200	200	200
Stock (100ng/ml)	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Vol of Stock (µL)	4	200	200	200	200	200	200	0
Final Conc (pg/ml)	1000	500	250	125	62.5	31.3	15.6	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. 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 90 min.**
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 30 min.**
5. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer. 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

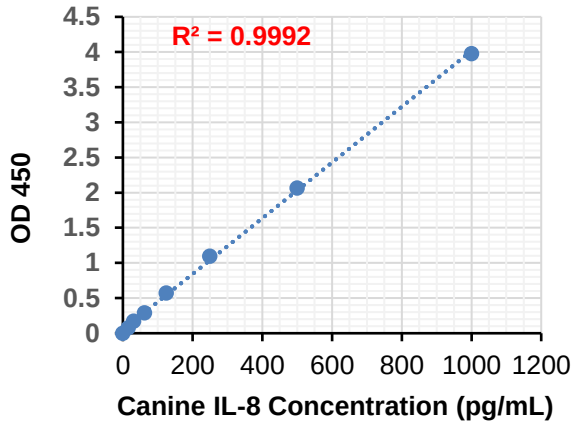
Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

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-8 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 (Fig 3. R^2=0.9992) is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Fig.3: Canine IL-8 Standard Curve



SENSITIVITY

The minimum detectable dose (MOD) of canine IL-8 is typically 7.8 pg/ml.

The Intra-assay CV is 3.8%

the Inter-assay CV is <10%.

SPECIFICITY

This assay recognizes natural and recombinant canine IL-8.

RELATIVE PRODUCTS

Canine IL-β ELISA (TBS33001)
Canine IL-4 ELISA (TBS33004)
Caine IL-5 ELISA (TBS33005)
Canine IL-6 ELISA (TBS33006)
Canine IL-12/23-P40 ELISA (TBS33012)
Canine IL-17A ELISA (TBS33017)

For research use only. Not for use in diagnostic procedure