Canine IL8 ELISA kit

Canine IL8 ELISA kit is an Enzyme Immunoassay kit for the quantification of Canine IL8 in serum, plasma and cell culture supernatants.

Catalog number: ARG82843

Package: 96 wells

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INTRODUCTION

Interleukin 8 (IL-8 or chemokine (C-X-C motif) ligand 8, CXCL8) is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells. Endothelial cells store IL-8 in their storage vesicles, the Weibel-Palade bodies. In humans, the interleukin-8 protein is encoded by the CXCL8 gene. IL-8 is initially produced as a precursor peptide of 99 amino acids which then undergoes cleavage to create several active IL-8 isoforms. In culture, a 72 amino acid peptide is the major form secreted by macrophages.

There are many receptors on the surface membrane capable of binding IL-8; the most frequently studied types are the G protein-coupled serpentine receptors CXCR1 and CXCR2. Expression and affinity for IL-8 differs between the two receptors (CXCR1 > CXCR2). Through a chain of biochemical reactions, IL-8 is secreted and is an important mediator of the immune reaction in the innate immune system response. [Provide by Wikipedia: IL-8]

PRINCIPLE OF THE ASSAY

This Canine IL8 ELISA kit is a quantitative sandwich enzyme immunoassay that measures the amount of Canine IL8 in the samples. An antibody specific for Canine IL8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL8 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Canine IL8 is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash

a substrate solution is added to the wells and color develops in proportion to the amount of IL8 bound in the initial step. The color development is stopped, and the intensity of the color is measured.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all reagent at 2-8°C upon receiving. Do not use kit components past kit expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C
20X Wash Buffer	50 mL	4°C
Standard	3 vial, lyophilized	4°C
Standard/Sample Diluent	20 mL	4°C
30X Detection Antibody	1 vial	4°C
Detection Antibody Diluent	16 mL	4°C
30X HRP Conjugate	1 vial	4°C
HRP Conjugate Diluent	16 mL	4°C
TMB Substrate	12 mL	4°C
Stop Solution	12 mL	4°C
Plate sealer	6 pic	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- 37°C Incubator
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is

blue before use, DO NOT USE IT.

- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2000 x g for 10 minutes at 4°C. Store frozen at-20°C or lower. Avoid freeze-thaw cycles.

Plasma: Collect blood with EDTA and centrifuge at 2000 x g for 10 minutes at 4°C. Store frozen at-20°C or lower. Avoid freeze-thaw cycles.

Cell culture supernatant: Centrifuge at 300 x g for 10 minutes at 4°C to remove the cell debris.

Note:

- Samples should be diluted with Standard/Sample Diluent and vortex for 1 minute prior to assay. If the OD value still exceeds the upper limit of the standard curve, further dilution is recommended till it falls in the detection range and the dilution factor must be used for calculation of the concentration.

- Do not use haemolytic, icteric or lipaemic specimens.
- Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer (E.g., add 50 mL of 20X Wash Buffer into 950 mL of distilled water to a final volume of 1000 mL).
- **1X Detection Antibody:** The 30X Detection Antibody should be stored at 4° for up to 1 months, if not used immediately. Centrifuge at 6000 x g for 1 minute to bring down the material prior to open the vial. Dilute 30X Detection Antibody into Detection Antibody Diluent to yield 1X Detection Antibody. 20 minutes before using, preparation the 1X Detection Antibody according to the amount required.
- **1X HRP Conjugate:** The 30X HRP Conjugate should be stored at 4° for up to 1 months, if not used immediately. Centrifuge at 6000 x g for 1 minute to bring down the material prior to open the vial. Dilute 30X HRP Conjugate into HRP Conjugate Diluent to yield 1X HRP Conjugate. 20 minutes before using, preparation the 1X Detection Antibody according to the amount required.

- **Standard:** The un-reconstituted standard can be stored at 4°C to-20°C for up to 6 months if not used immediately. Centrifuge at 6000 x g for 1 minute to bring down the material prior to open the vial. Add 1000 µL of Standard/Sample Diluent to a Standard vial to make the high standard concentration of 800 pg/ml and vortex 20 seconds and allow it to sit for 15 minutes. Diluted the standard as follow.

Standard tube	IL-8 (pg/mL)	1X Assay Buffer (µL)	Standard stock, 800 pg/mL (µL)
S1	800	0	500
S2	400	250	250 of S1
S3	200	250	250 of S2
S4	100	250	250 of S3
S5	50	250	250 of S4
S6	25	250	250 of S5
S7	12.5	250	250 of S6
S0	0	500	0

Note: Working standard should be prepared immediately prior to use.

ASSAY PROCEDURE

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. It is recommended that all samples and standards be assayed in duplicate.

1. Vortex the standards and samples for **10 seconds** before applying to the plate. Add **100 µL** of **diluted samples, Blank (Standard/Sample Diluent as Blank)** or **each diluted Standard** into respective wells.
2. Cover the plate and incubate for **90 minutes** at **37ºC** in the dark.
3. Aspirate each well and wash, repeating the process 4 time for a total 5 washes. Wash by filling each well with **1X Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **Working Dilution of Detection Antibody** to each well. Mix well by repeated pipetting.
5. Cover the plate and incubate for **60 minutes** at **37ºC** in the dark.
6. Aspirate and wash plate as in step 3.
7. Add **100 µL** of **Working Dilution of HRP Conjugate** to each well.
8. Cover the plate and incubate for **30 minutes** at **37ºC** in the dark.
9. Aspirate and wash plate as in step 3.
10. Add **100 µL** of **TMB Substrate** in each well.
11. Incubate for **15 minutes** at **37ºC** in the dark.
12. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
13. Read the absorbance with a plate reader at **O.D. 450 nm** within **3 minutes.**

CALCULATION OF RESULTS

1. Subtract Blank value from all standards and samples to determine corrected absorbance.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

6.25 pg/mL

Precision

Intra Assay CV: < 10%; Inter Assay CV: < 10%