ab155465 – Interleukin-8 (IL-8) Canine ELISA Kit

Instructions for Use

For the quantitative measurement of Interleukin-8 (IL-8) in canine serum, plasma and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

Version 2a Last Updated 25 February 2013

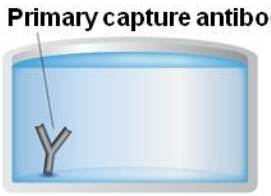
Table of Contents

1. BACKGROUND

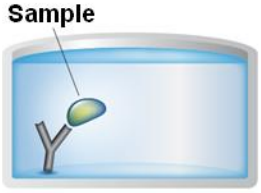
Abcam's Interleukin-8 (IL-8) Canine ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of canine Interleukin-8 (IL-8) in serum, plasma and cell culture supernatants.

This assay employs an antibody specific for canine IL-8 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-8 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-canine IL-8 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IL-8 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

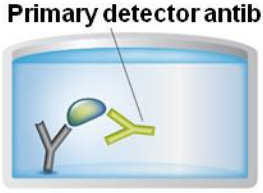
2. ASSAY SUMMARY



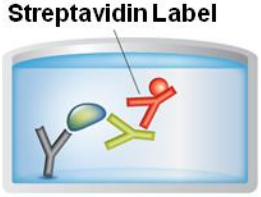
Prepare all reagents, samples and standards as instructed.



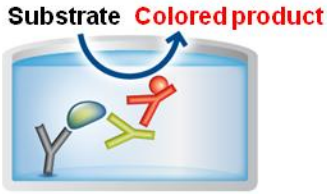
Add standard or sample to each well used. Incubate at room temperature



Add prepared biotin antibody to each well. Incubate at room temperature.



Add prepared Streptavidin solution. Incubate at room temperature.



Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
IL-8 Microplate (12 x 8 well strips)	96 wells	-20ºC
20X Wash Buffer Concentrate	25 mL	-20ºC
IL-8 Canine Standard (recombinant)	2 vials	-20ºC
5X Assay Diluent	15 mL	-20ºC
Detection Antibody IL-8 (biotinylated anti-Canine IL-8)	2 vials	-20ºC
200X HRP-Streptavidin concentrate	200 µL	-20ºC
TMB One-Step Substrate Reagent	12 mL	-20ºC
Stop Solution	8 mL	-20ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps. When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder

thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.

- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPERATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 **1X Assay Diluent**

Dilute 5X Assay Diluent 5-fold with deionized or distilled water before use.

9.2 **20X Wash Solution**

If the 20X Wash Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.3 **Detection Antibody IL-8 (biotinylated anti-Canine IL-8)**

Briefly spin the Detection Antibody vial before use. Add 100 µL of 1X Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent and used in Assay Procedure.

9.4 **1X HRP-Streptavidin Solution**

Briefly spin the 200X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. The 200X HRP-Streptavidin concentrate should be diluted 200-fold with 1X Assay Diluent.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 50 µL of HRP-Streptavidin concentrate into a tube with 10 mL 1X Assay Diluent to prepare a 200-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

10. STANDARD PREPARATIONS

- **Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.**
- **Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.**

10.1 Briefly spin the vial of IL-8 Canine Standard and then add 400 µL 1X Assay Diluent into the IL-8 Canine Standard vial to prepare a 50 ng/mL standard (**Stock Standard**).

10.2 Ensure the powder is thoroughly dissolved by gentle mixing.

10.3 Label tubes #1-8.

10.4 Add 15 µL Stock Standard into tube #1 with 485 µL 1X Assay Diluent to prepare a 1,500 pg/mL standard solution = **Standard #1** (see table below).

10.5 Add 300 µL 1X Assay Diluent into remaining tubes.

10.6 Add 200 µL Standard #1 to tube #2 and mix thoroughly = **Standard #2.**

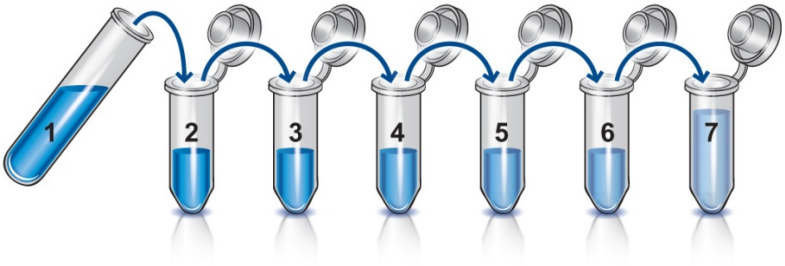
10.7 Transfer 200 µL from tube #2 to #3, mix thoroughly = **Standard #3.**

10.8 Using the table below as a guide, prepare further serial dilutions.

10.9 1X Assay Diluent serves as the zero standard 0 ng/mL (tube #8).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	15	485	500	50,000	1,500
2	200	300	500	1,500	600
3	200	300	500	600	240
4	200	300	500	240	96
5	200	300	500	96	38.4
6	200	300	500	38.4	15.4
7	200	300	500	15.4	6.14
8	0	300	300	0	0



11. SAMPLE PREPARATION

General Sample Information:

- Sample dilution: If your samples need to be diluted, 1X Assay Diluent should be used for dilution of serum/plasma/culture supernatants.
- Suggested dilution for normal serum/plasma: 10-100 fold.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1. Add 100 µL of each standard (see Standard Preparations, section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.

13.2. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or autowasher. 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.

13.3. Add 100 µL of 1X prepared biotinylated antibody (see Reagent Preparation, section 9) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4. Discard the solution. Repeat the wash as in step 13.2.

13.5. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation, section 9) to each well. Incubate for 45 minutes at room temperature with gentle shaking

13.6. Discard the solution. Repeat the wash as in step 13.2.

13.7. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

13.8. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

14. CALCULATIONS

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

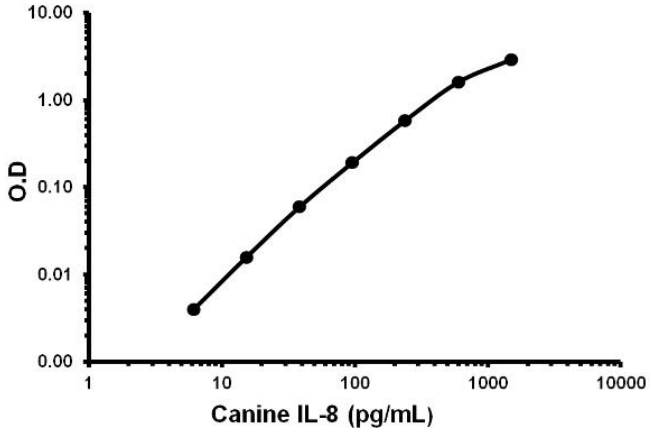
Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four- parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4- parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements	
Conc. (pg/mL)	**O.D.**
6.14	0.00
15.36	0.02
38.40	0.06
96.00	0.19
240.00	0.59
600.00	1.61
1,500.00	2.92

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of IL-8 is typically less than 6 pg/mL.

RECOVERY –

Recovery was determined by spiking various levels of IL-8 into normal canine serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	110.2	102-118
Plasma	103.2	84-122
Cell culture media	128.2	122-132

LINEARITY OF DILUTION -

Serum Dilution	Average % Expected Value	Range (%)
1:2	117.9	110-126
1:4	107.8	100-116

Plasma Dilution	Average % Expected Value	Range (%)
1:2	113.6	107-119
1:4	113.4	94-129

Cell Culture Media Dilution	Average % Expected Value	Range (%)
1:2	100.6	93-109
1:4	73.96	67-81

PRECISION –

	Intra-Assay	Inter-Assay
%CV	<10%	<12%

17. ASSAY SPECIFICITY

This ELISA pair antibody detects canine IL-8. Other species not determined yet.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation time; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.
	Stop solution	Stop solution should be added to each well before measuring

19. NOTES

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)