IgE (Canine) ELISA

For the quantitative determination of IgE in canine serum or plasma

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	41-IGECA-E01
Size:	96 wells
Version:	4.1 - ALPCO 2.0

INTENDED USE

This total Canine IgE test kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for the determination of IgE in canine serum or plasma.

PRINCIPLE OF THE ASSAY

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the IgE present in samples reacts with the anti-IgE antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-IgE antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound IgE. Following another washing step, the bound enzyme is assayed by the addition of a chromo-genic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of IgE in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of IgE in the test sample. The quantity of IgE in the test sample can be interpolated from the standard curve constructed from the standards and corrected for sample dilution.

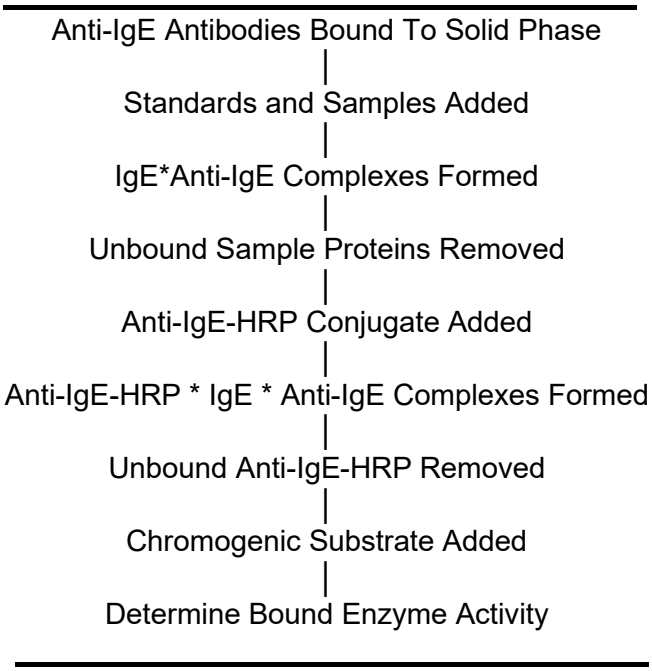

Figure 1.

LIMITATION OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipetting, washing technique, incubation time or temperature. Do not mix or substitute reagents with those from other lots or sources.

KIT COMPONENTS

The expiration date for the kit and its components is stated on the box label. All components should be stable up to the expiration date if stored and used per this kit protocol insert.

Component	Description	Preparation	Storage	Stability
ELISA Microplate, antibody coated	One plate of 12 removable 8 well strips, antibody coated	Ready to use as supplied	2-8 °C, In sealed foil bag with desiccant	With proper storage the plate strips are stable until the expiration date.
Enzyme Conjugated Detection Antibody	One vial of 150 µL of 100X Horseradish Peroxidase Conjugated antibody in a stabilizing buffer	Dilute 1:100 immediately prior to use.	2-8 ° C in the dark	The working conjugate solution should be diluted immediately prior to use. The 100X conjugate is stable until the expiration date.
Calibrator	One vial of calibrator	Refer to the Certificate of Analysis (COA).	2-8 ° C for lyophilized calibrator. Aliquoted and frozen if reconstituted. Avoid multiple freeze-thaw cycles.	The working standard solutions should be prepared immediately prior to use.
Diluent Concentrate	One 50 mL bottle of 5X diluent buffer	Dilute 1:5 to make 1X working solution.	2-8 ° C for both 1X working solution and 5X concentrate	The 1X working solution is stable for at least one week from the date of preparation. The 5X concentrate is stable until the expiration date.
Wash Solution Concentrate	One 50 mL bottle of 20X wash solution	Dilute 1:20 to make 1X working solution.	2-8 ° C for both 1X working solution and 20X concentrate	The 1X working solution is stable for at least one week from the date of preparation. The 20X concentrate is stable until the expiration date.
Chromogen-Substrate Solution	One bottle of 12 mL 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.	Ready to use as supplied	2-8 ° C in the dark	Protect from light. The Substrate Solution is stable until the expiration date.
STOP Solution <u>WARNING: Avoid Contact with Skin</u>	One 12 mL bottle of 0.3 M sulfuric acid.	Ready to use as supplied	2-8 ° C	The Stop Solution is stable until the expiration date.

MATERIAL REQUIRED BUT NOT PROVIDED

- Precision pipette (2 µl to 100 µl) for making and dispensing dilutions
- Test tubes
- Microtiter washer/aspirator or squirt bottle
- Distilled or Deionized H_2O
- Microtiter Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Centrifuge for sample collection
- Anticoagulant for plasma sample collection

SPECIMEN COLLECTION AND HANDLING

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing. If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution. The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum samples - Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at -80^0 C (preferably) or -20^{0}C. Avoid repeated freeze-thaw cycles.

Plasma samples - Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at -80^0 C (preferably) or -20^0 C. Avoid repeated freeze-thaw cycles.

Known interfering substances - Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

DILUTION OF SAMPLES

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

Serum & Plasma samples — Recommended starting dilution is 1:2,000. To prepare a 1:2,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This yields a 1:100 dilution. Next, dilute the 1:100 by transferring 20 µL into 380 µL of 1X diluent. This yields a 1:2,000 dilution. Mix thoroughly at each stage.

REAGENT PREPARATION

Bring all reagents to room temperature (16^{0}C to 25^{0}C) before use.

Diluent Concentrate - The Diluent Solution supplied is a 5X Concentrate and must be diluted 1:5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_20).

Wash Solution Concentrate - The Wash Solution supplied is a 20X Concentrate and must be diluted 1:20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_20). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35^{0}C before dilution can dissolve crystals.

Enzyme-Antibody Conjugate - Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

Pre-coated ELISA Microplate - Ready to use. Unseal foil pouch and remove plate from pouch. Remove and place all unused strips and wells back in pouch and re-seal along with desiccant.

Dog IgE Calibrator — Prepare according to the lot specific Certificate of Analysis.

ASSAY PROCEDURE

1. All samples and standards should be assayed in duplicate.

2. The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

Pipette 100 µL of

- Standard 0 (0.0 ng/mL) in duplicate
- Standard 1 (6.25 ng/mL) in duplicate
- Standard 2 (12.50 ng/mL) in duplicate
- Standard 3 (25 ng/mL) in duplicate
- Standard 4 (50 ng/mL) in duplicate
- Standard 5 (100 ng/mL) in duplicate
- Standard 6 (200 ng/mL) in duplicate
- Standard 7 (400 ng/mL) in duplicate

3. Pipette 100 µl of sample (in duplicate) into predesignated wells.

4. Incubate the microtiter plate at room temperature for thirty (30 ± 2) minutes. Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with 1X working Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with 1X working Wash Solution, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty (30 ± 2) minutes. Keep plate covered in the dark and level during incubation.

8. Wash and blot the wells as described in Steps 5/6.

9. Pipette 100 µL of TMB Substrate Solution into each well.

10. Incubate in the dark at room temperature for precisely ten (10) minutes.

11. After ten minutes, add 100 µL of Stop Solution to each well.

12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes using a calibrated plate reader.

CALCULATION OF RESULTS

1. Subtract the average background value (average absorbance of standard zero) from all test values.

2. Average the duplicate readings for each standard and use the results to construct a standard curve. Construct the standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve fit. Second order polynomial (quadratic) or other curve fits may also be used but will less precisely fit the data.

3. Interpolate test sample values from standard curve. Correct for dilution factor to arrive at the IgE concentration in original samples.