Canine IgE

Immunoperoxidase Assay for Determination of Dog IgE in Dog serum/plasma.

Directions for Use

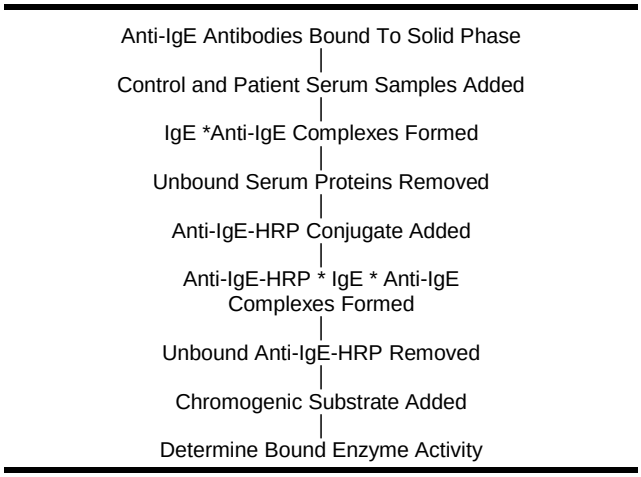
Version 1.0

INTENDED USE

The total Dog IgE test kits are a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring IgE in serum or plasma of Dogs.

PRINCIPLE OF THE ASSAY

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the IgE present in serum sample reacts with the anti-IgE antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound serum proteins by washing, anti-IgE antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound serum IgE. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic 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 serum dilution.



REAGENTS

(Quantities sufficient for 96 determinations)

1. DILUENT CONCENTRATE
One bottle containing 50 mL of a 5X concentrated phosphate buffered saline (PBS) solution containing bovine serum albumin, 0.25% Tween, and 0.1% Proclin 300 as a preservative.

2. WASH SOLUTION CONCENTRATE
One bottle containing 50 ml of a 20X concentrated phosphate buffered saline (PBS) solution containing 1% Tween.

3. ENZYME-ANTIBODY CONJUGATE
One vial containing 200 µL of 100X concentrated affinity purified anti-Dog IgE antibody conjugated with horseradish peroxidase in a stabilizing buffer.

4. CHROMOGEN-SUBSTRATE SOLUTION
One vial containing 12 mL of 3,3',5,5'-tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

5. STOP SOLUTION
One vial containing 12 ml 0.3 M sulfuric acid.

WARNING: Avoid contact with skin.

6. ANTI-DOG IgE ELISA MICRO PLATE
Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Dog IgE.

7. DOG IgE CALIBRATOR
One vial containing 0.2 ml of Dog IgE calibrator.

FOR IN VITRO USE ONLY

REAGENT PREPARATION

1. DILUENT CONCENTRATE
The Diluent Solution supplied is a 5X Concentrate and must be diluted 1:5 with distilled or deionized water.

2. WASH SOLUTION CONCENTRATE
The Wash Solution supplied is a 20X Concentrate and must be diluted 1:20 with distilled or deionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. ENZYME-ANTIBODY CONJUGATE
The required amount of working conjugate solution for each microtitre plate is prepared by adding 100 µL of 100X concentrated Enzyme-Antibody Conjugate to 10 mL of Diluent. Mix uniformly, but gently. Avoid foaming.

4. CHROMOGEN-SUBSTRATE SOLUTION
Ready to use as supplied.

5. STOP SOLUTION
Ready to use as supplied.

6. ANTI-DOG IgE ELISA MICRO PLATE
Ready to use as supplied.

7. DOG IgE STANDARDS
The calibrator is now at a concentration of 24 µg/ml. Dog IgE standards need to be prepared immediately prior to use (see chart below). Mix well between each step. Avoid foaming.

Standard	ng/ml	Volume added to 1x Diluent	Volume of 1x Diluent →
1	800	20 µl Dog IgE Calibrator	580 µl
2	400	300 µl standard 1	300 µl
3	200	300 µl standard 2	300 µl
4	100	300 µl standard 3	300 µl
5	50	300 µl standard 4	300 µl
6	25	300 µl standard 5	300 µl

STORAGE AND STABILITY

The expiration date for the package is stated on the box label.

1. DILUENT
The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8°C.

2. WASH SOLUTION
The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.

3. ENZYME-ANTIBODY CONJUGATE
100X concentrated horseradish peroxidase anti-Dog IgE conjugate should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for one day.

4. CHROMOGEN-SUBSTRATE SOLUTION
The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.

5. STOP SOLUTION
The Stop Solution should be stored at 4-8°C and is stable until the expiration date.

6. ANTI-DOG IgE ELISA MICRO PLATE
Anti-Dog IgE coated wells are stable until the expiration date, and should be stored at 4-8°C in the sealed foil pouch with desiccant pack.

7. DOG IgE CALIBRATOR
The Dog IgE Calibrator should be stored at 4C. The working standard solutions should be prepared immediately prior to use and are stable for 1 day.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the standard solutions should be within 20 % of the expected values.

SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture and the serum separated from the cells, after clot formation, by centrifugation. Specimens may be shipped at room temperature and then stored refrigerated at 2-8°C if testing is to take place within one week after collection. If testing is to take place later than one week, specimens should be stored at -20°C. Avoid repeated freeze-thaw cycles.

1. Precautions

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds. This kit should only be used by qualified technicians.

2. Additives and Preservatives

No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

3. Known interfering substances

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

MATERIAL PROVIDED
See "REAGENTS"

MATERIALS REQUIRED
BUT NOT PROVIDED

- Precision pipette (2 μL to 200 μL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H_2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Vortex mixer

ASSAY PROTOCOL

DILUTION OF SERUM SAMPLES
The assay for quantification of total IgE in serum requires that each test sample be diluted before use. A 1:2,000 dilution of serum is recommended for most samples. **For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required**.

1. To prepare a 1:2,000 dilution of sample, transfer 5 μL of sample to 495μL of diluent. This gives you a 1:100 dilution. Next, dilute the 1:100 dilution by transferring 20μL to 380 μL of dilutent. This gives you a 1:2,000 dilution. Mix thoroughly at each step.

PROCEDURE

Bring all reagents to room temperature before use.

1. Add 100 μL of Diluent to each of the wells in 1A & 2A. These will serve for an evaluation of the background associated with the assay.

2. Pipette 100 μL of
   Standard 1 (800 ng/ml) into wells 1B & 2B
   Standard 2 (400 ng/ml) into wells 1C & 2C
   Standard 3 (200 ng/ml) into wells 1D & 2D
   Standard 4 (100 ng/ml) into wells 1E & 2E
   Standard 5 (50 ng/ml) into wells 1F & 2F
   Standard 6 (25 ng/ml) into wells 1G & 2G

3. Pipette 100 μL of diluted serum sample (test sample 1) into wells 3A & 4A. The next sample goes in wells 3B & 4B, the next in 3C & 4C and so on.

4. Incubate the micro titer plate at 22°C (room temperature) for thirty (30 ± 2) minutes. Keep plate level during incubation.

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

6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, 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 in dark at 22°C (room temperature) for thirty (30 ± 2) minutes.

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

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

10. Incubate in 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. Calibrate the plate reader to air.

STABILITY OF THE FINAL REACTION MIXTURE

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.

2. Using the results observed for the standards construct a Standard Curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

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

QUALITY CONTROL

In accord with good laboratory practice, the Assays for total IgE require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

LIMITATION OF THE PROCEDURE

1. 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.

2. 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 pipettings.