# Dog Immunoglobulin-E (IgE) ELISA
# Life Diagnostics, Inc., Catalog Number: IGE-4

## INTRODUCTION

The dog IgE ELISA kit is designed for measurement of IgE in dog serum or plasma. The assay uses a monoclonal dog IgE antibody (IGE-4-4D1) for solid phase (microtiter wells) immobilization, and a horseradish peroxidase (HRP) conjugated dog IgE monoclonal antibody (IGE-4-13H5) for detection.

## PRINCIPLE OF THE TEST

Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside dog IgE standards. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. IgE molecules are thus sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies. TMB Reagent is added and incubated for 45 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow. Optical density is measured spectrophotometrically at 450 nm. The concentration of IgE is proportional to the optical density of the test sample and is derived from a standard curve.

## MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti dog IgE coated 96-well plate (12 strips of 8 wells)
- HRP Conjugate Reagent, 11 ml
- Reference standard (lyophilized)[1]
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD50-1, 50 ml
- TMB Reagent (One-Step): TMB11-1, 11 ml
- Stop Solution (1N HCl): SS11-1, 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of 150 rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450nm
- Graph paper (PC graphing software is optional)

## STORAGE

The test kit will remain stable for six months from the date of purchase provided that the components are stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

## GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (25°C) before use.
3. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

## WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

## STANDARD PREPARATION

1. The dog IgE standard is provided as a lyophilized stock. Reconstitute as described on the vial label. *(the reconstituted standard is stable at 4°C for at least 24 hours but should be aliquoted and frozen at -20°C after reconstitution if future use is intended)*.
2. Label 8 polypropylene or glass tubes as 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 and 0 ng/ml.
3. Into the tube labeled 50 ng/ml, pipette the volume of diluent detailed on the IgE standard vial label. Then add the indicated volume of IgE standard (shown on the IgE standard vial label) and mix gently. This provides the 50 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 25, 12.5, 6.25, 3.13, 1.56 and 0.78 and 0 ng/ml.
5. Prepare a 25 ng/ml standard by diluting and mixing 250 µl of the 50 ng/ml standard with 250 µl of diluent in the tube labeled 25 ng/ml.
6. Similarly prepare the 12.5 to 0.78 ng/ml standards by serial dilution.

## SAMPLE PREPARATION

In studies at Life Diagnostics, we found IgE levels ranging from 15 to 88 µg/ml in normal Beagle serum 38.4±29.2 µg/ml (mean±SD, n = 5). Nimmo Wilkie et. al.[1] reported IgE levels ranging from 24-410 ug/ml in normal, random sourced, dogs. To obtain values within range of the standard curve, we suggest that samples initially be diluted 4,000-fold using the following procedure for each sample to be tested.

1. Dispense 98 µl and 247.5 µl of diluent into separate tubes.
2. Pipette and mix 2.0 µl of the serum/plasma sample into the tube containing 98 µl of diluent. This provides a 50-fold diluted sample.
3. Mix 2.5 µl of the 50-fold diluted sample with the 247.5 µl of diluent in the second tube. This provides a 4,000-fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

## ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate in an incubator shaker at 150 rpm and 25°C for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 µl/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.

[1] The reference standard consists of lyophilized dog serum of known IgE concentration in a BSA matrix. The IgE concentration was determined relative to purified dog IgE obtained from an independent laboratory.

6. Add 100 µl of enzyme conjugate reagent into each well.
7. Incubate in an incubator shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed in steps 4 to 5 above.
9. Dispense 100 µl of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
13. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

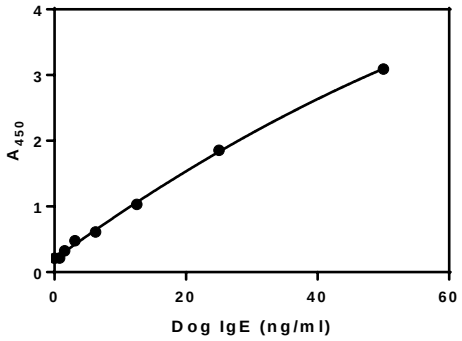
## CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus the concentration.
2. Fit the standard curve to a second order polynomial (quadratic) equation/model and determine the concentration of the samples from the standard curve.
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the plasma sample.
4. If the $A_{450}$ values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

## TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y-axis against IgE concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.

| IgE (ng/ml) | $A_{450}$ |
|---|---|
| 50 | 3.093 |
| 25 | 1.855 |
| 12.5 | 1.032 |
| 6.25 | 0.612 |
| 3.13 | 0.479 |
| 1.56 | 0.325 |
| 0.78 | 0.216 |
| 0.0 | 0.211 |




## LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above.
2. We perform validation and quality control testing of our ELISA kits using shaking incubators set at 150 rpm and 25°C. Testing at lower speeds and temperatures will likely result in slightly lower absorbance values.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

## REFERENCES

1. Nimmo Wilkie JS, Yager JA, Eyre P and Parker WM. Morphometric analyses of the skin of dogs with atopic dermatitis and correlations with cutaneous and plasma histamine and total serum IgE. Vet Pathol. 27:179-186 (1990)

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