Dog IgA ELISA
Life Diagnostics, Inc., Catalog Number: IGA-4

INTRODUCTION

The dog IgA ELISA kit is designed for measurement of IgA in serum or plasma. The assay uses goat anti-dog IgA for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated goat anti-dog IgA antibodies for detection. Both capture and detection antibodies were cross absorbed on dog IgG and IgM agarose columns, thereby ensuring specificity for IgA. Cross-reactivity with immunoglobulins from other species has not been investigated.

PRINCIPLE OF THE ASSAY

Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside dog IgA standards. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. IgA 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 20 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 IgA 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 IgA coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- IgA stock (lyophilized)
- 20x Wash Solution: TBS50-20, 50 ml
- 10x Diluent: RD25-10, 25 ml
- TMB: TMB11-1, 11 ml
- Stop Solution: 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
- PC graphing software

STORAGE

The test kit will remain stable for six months from the date of purchase provided that the components are stored at 2°C-8°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 before use.
3. Optimal results are achieved if all reagents are pipetted into the wells of the microtiter plate within 5 minutes.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use, estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

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 IgA standard is provided as a lyophilized stock. Reconstitute as described on the vial label *(the reconstituted standard should be frozen at or below -20°C if future use is intended)*.
2. Label 7 polypropylene or glass tubes as 250, 125, 62.5, 31.25, 15.63, 7.81 and 3.91 ng/ml.
3. Into the tube labeled 250 ng/ml, pipette the volume of diluent detailed on the IgA stock vial label. Then add the indicated volume of IgA stock and mix gently. This provides the 250 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 125, 62.5, 31.25, 15.63, 7.81 and 3.91 ng/ml.
5. Prepare a 125 ng/ml standard by diluting and mixing 250 µl of the 250 ng/ml standard with 250 µl of diluent in the tube labeled 125 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

General Note: We found that IgA was present in dog serum at concentrations of 0.5 - 4 mg/ml. To obtain values within range of the standard curve, we suggest that samples initially be diluted 10,000-fold using the following procedure for each sample to be tested:

1. Dispense 247.5 µl of 1x diluent into two tubes.
2. Pipette and mix 2.5 µl of serum/plasma with 247.5 µl of diluent in the first tube. This provides a 100-fold diluted sample.
3. Mix 2.5 µl of the 100-fold diluted sample with 247.5 µl of diluent in the second tube. This provides a 10,000-fold dilution.

To avoid matrix effects, serum and plasma should be diluted at least 4,000-fold. Fluids other than serum or plasma will likely have lower IgA levels than those found in serum. Optimal dilutions of such samples should be determined empirically.

Life Diagnostics, Inc., P.O. Box 5205, West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

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 on an orbital plate shaker at 150 rpm and 25°C for 45 minutes.
4. Aspirate the contents of the wells and wash five 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 buffer.
6. Add 100 µl of HRP conjugate reagent into each well.
7. Incubate on an orbital plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 µl of TMB into each well.
10. Incubate on an orbital plate shaker at 150 rpm and 25°C for 20 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.

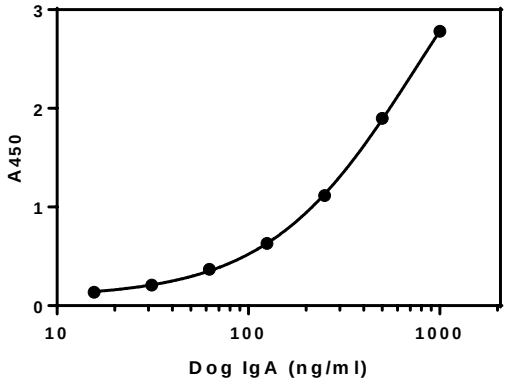
CALCULATION OF RESULTS

1. Using graphing software, construct a standard curve by plotting the absorbance values for the standards versus the $\log_{10}$ of the IgA concentration and fit the data to a four-parameter logistic equation.
2. Derive the corresponding concentration of IgA in the samples from the standard curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgA in the sample.
4. If the absorbance values of diluted samples fall outside the standard curve, the original samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450 nm on the Y-axis against $\log_{10}$ IgA concentrations on the X-axis is shown below. This curve is for the purpose of illustration only. It should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

IgA (ng/ml)	A_{450}
250	2.781
125	1.898
62.5	1.117
31.25	0.632
15.63	0.368
7.81	0.209
3.91	0.136



Rev 102519

For technical assistance please email us at techsupport@lifediagnostics.com