## Introduction

The CellTrend IgA(dog)-ELISA is designed for the quantitative determination of dog IgA in complex samples (serum or other biological samples).

## Principle of the Assay

The determination of dog IgA is carried out as direct sandwich ELISA. An antibody specific for dog IgA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgA present is bound. After washing away any unbound substances, an enzyme-linked antibody is added. Following a wash, a substrate solution is added to the wells and color develops in proportion to the amount of antibody conjugate. The absorption at 450 nm is proportional to the IgA concentration.

## Precautions

Store the kit at 2-8 °C.

For research use only. Not for use in diagnostic procedures.

For in vitro use only.

Do not use the reagents beyond the expiration date marked on box label.

Please read the instructions carefully before using the kit.

Do not mix reagents from different lots.

Some components of this kit contain Thimerosal, a mercury containing compound. The stop solution contains 0.5 M sulphuric acid. Follow routine precautions for handling hazardous chemicals.

## Other supplies required

Deionized or distilled water
Graduated cylinder
Micropipettes, multipipette
Microplate reader

## Preparation of reagents and samples

- Bring all reagents to room temperature before use. If crystals have formed, mix gently until the crystals have completely dissolved.

- The microplate strips are ready to use. Remove excess strips (breakable) from the frame, reseal in the plastic bag with the desiccant and store at 2-8 °C

- Dilute the wash buffer with deionized or distilled water **1:10** (e. g. 50 ml + 450 ml water). The diluted solution is stable for 30 days at 2-8 °C.

- Dilute the HRP conjugate with diluent **1:100** (e. g. 50 µl + 4950 µl diluent). The required amount of conjugate solution should be prepared freshly.

- Use the Standard concentrate to produce a 1:2-dilution series with diluent (e. g. 250 µl + 250 µl diluent):

| standard | preparation | conc. ng/ml |
|---|---|---|
| S 7 | standard conc. undiluted | 1000.000 |
| S 6 | S 7 1:2 diluted | 500.000 |
| S 5 | S 6 1:2 diluted | 250.000 |
| S 4 | S 5 1:2 diluted | 125.000 |
| S 3 | S 4 1:2 diluted | 62.500 |
| S 2 | S 3 1:2 diluted | 31.250 |
| S 1 | S 2 1:2 diluted | 15.625 |

Dilute the samples with diluent. To exclude matrix effects the dilution factor should be at least 1:10. If samples generate values outside the standard curve, the dilution factor may be varied.

## Assay procedure

It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, standard curve and samples as directed in the previous section.
2. Pipette 100 µl of samples, standards, or diluent (as negative control) into the wells.
3. Seal wells with adhesive strip and incubate for 1 hour at room temperature with shaking (recommended) or 2 hours without shaking.
4. Aspirate fluid from wells and wash three times with 300 µl wash buffer. After the last wash, invert the plate and tap on a clean paper towel.
5. Add 100 µl of HRP conjugate to each well.
6. Seal wells with adhesive strip and incubate for 1 hour at room temperature with shaking (recommended) or 2 hours without shaking.
7. Repeat the wash as in step 4.
8. Dispense 100 µl of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature in the dark.
10. Add 100 µl of stop solution to each well.
11. Determine the absorbance within 30 minutes at 450 nm. A reference wavelength of 620 nm/690 nm is recommended.

## Calculation of results

Create a standard curve using computer software capable of generating a curve fit. As an alternative, draw a standard curve on semi-log paper (x-axis: log, IgA concentration; y-axis: linear, absorbance). The IgA concentrations can be calculated from the standard curve. The calculated concentrations must be multiplied by the sample dilution factor.

If the absorbance of some samples is outside the standard curve a subsequent determination with changed samples dilutions will provide a proper result.

**Materials provided:**

| Number of determinations/Catalog No. | 1x96 Determ.<br>52400 |
|---|---|
| Microplate strips, antibody coated | 12 x 8 |
| Wash buffer, 10fold conc. ✦ | 50 ml |
| Diluent, ready to use ✦ | 100 ml |
| Standard concentrate, 1000 ng/ml ✦ | 2 ml |
| Anti-IgA(dog)-Ab., HRP conjugate, 100fold conc. ✦ | 0,2 ml |
| TMB substrate, ready to use | 12 ml |
| Stop solution, ready to use (0.5 M sulphuric acid) | 12 ml |

✦: contains Thimerosal

**Assay procedure summary:**

A. Preparation

1. Bring all reagents to room temperature
2. Dilute wash buffer 1:10
3. Prepare the standard curve from a 1:2-dilution series of standard concentrate with diluent
4. Dilute samples with diluent
5. Dilute freshly HRP conjugate 1:100 with diluent

B. Performance

1. Pipette 100 µl of samples, standards, diluent (blank) into the wells
2. Incubate for 1 hour at room temperature with shaking (or 2 hrs without shaking)
3. Wash three times with 300 µl of wash buffer
4. Add 100 µl of HRP conjugate to each well
5. Incubate for 1 hour at room temperature with shaking (or 2 hrs without shaking)
6. Wash three times with 300 µl of wash buffer
7. Dispense 100 µl of TMB substrate solution
8. Incubate for 15 minutes at room temperature in the dark
9. Add 100 µl of stop solution
10. Measure absorption at 450 nm

**INSTRUCTIONS FOR USE**

# ELISA for Quantitative Determination of IgA (Dog)

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