Cat IgG ELISA

INTRODUCTION

The cat IgG ELISA kit is designed for measurement of IgG in cat serum or plasma. The assay uses goat anti-cat IgG for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated goat anti-cat IgG antibodies for detection. Both capture and detection antibodies react specifically with the Fc portion of cat IgG. Cross-reactivity with immunoglobulins from other species has not been investigated.

PRINCIPLE OF THE TEST

Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside cat IgG standards. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. IgG molecules are thus sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and 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, and optical density is measured spectrophotometrically at 450 nm. The concentration of IgG 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 Cat IgG Coated 96-well Plate (12 strips of 8 wells)
- HRP Conjugate Reagent, 11 ml
- Reference Standard (lyophilized)
- 20x Wash Solution, 50 ml
- 10x Immunoglobulin Diluent, 25 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 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 450 nm
- Graph paper (PC graphing software is optional)

STORAGE OF THE TEST KIT

The test kit will remain stable for six months from the date of purchase provided that the components are stored at 2-8°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 (18-25°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.

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 cat IgG standard is provided as a lyophilized stock. Reconstitute with 1.0 ml of distilled or deionized water ***(the reconstituted standard is stable at 4°C for one week 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, 6.3, 3.1, 1.6, 0.8 and 0 ng/ml.
3. Into the tube labeled 50 ng/ml, pipette the volume of diluent detailed on the IgG standard vial label. Then add the indicated volume of IgG standard (shown on the IgG standard vial label) and mix gently. This provides the 50 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes 25, 12.5, 6.3, 3.1, 1.6, 0.8 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 12.5, 6.3, 3.1, 1.6, 0.8 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: IgG is typically present in cat serum or plasma at concentrations of ~15 mg/ml. In order to obtain values within range of the standard curve, we suggest that samples initially be diluted 800,000 fold using the following procedure for each sample to be tested:

1. Dispense 999 µl and 799 µl of 1x diluent into separate tubes.
2. Pipette and mix 1.0 µl of the serum/plasma sample into the tube containing 999 µl of diluent. This provides a 1000 fold diluted sample.
3. Mix 1.0 µl of the 1000 fold diluted sample with the 799 µl of diluent in the second tube. This provides an 800,000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

Tissue extracts and body fluids other than serum or plasma will likely have lower IgG levels than those found in serum. Optimal dilutions of such samples should be determined empirically.

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 micro-plate shaker at 100-150 rpm at room temperature (18-25°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.
6. Add 100 µl of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°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 (18-25°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*.

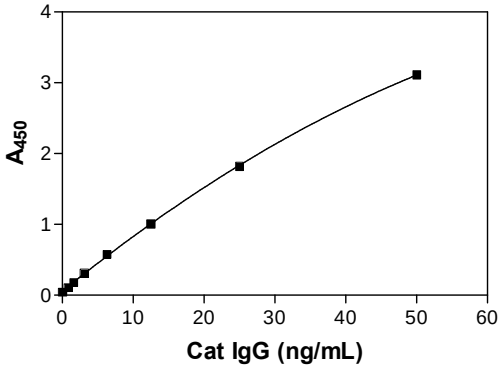
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgG in ng/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgG in the sample.
5. PC graphing software may be used for the above steps. We recommend a fit using a second order polynomial equation.
6. If the OD_{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 450 nm on the Y-axis against IgG 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. Each user should obtain his or her data and standard curve in each experiment.

IgG (ng/ml)	A_{450}
50	3.111
25	1.823
12.5	1.006
6.3	0.580
3.1	0.313
1.6	0.181
0.8	0.108
0.0	0.044



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. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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For technical assistance please email us at
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