Goat IgG ELISA
Life Diagnostics, Inc., Catalog Number: IGG-13

INTRODUCTION

This ELISA kit is designed for measurement of IgG in goat serum and plasma. The assay uses sheep anti-goat IgG for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated sheep anti-goat IgG for detection. Both capture and detection antibodies react specifically with goat IgG. 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 goat 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. After washing the wells to remove unbound HRP-conjugate, TMB reagent is added and incubated for 20 minutes. 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 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-goat IgG coated 96-well plate — Store ≤ -20°C
- HRP conjugate stock, 50 µl — Store ≤ -20°C
- Goat IgG stock (lyophilized), 3 vials — Store ≤ -20°C
- 20x Wash solution: TBS20-50 50 ml
- 10x Diluent: CSD25-10, 25 ml
- TMB reagent: TMB-11, 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
- Plate washer
- Plate reader with an OD range of 0-4 at 450 nm
- Curve fitting software

STORAGE

The kit will remain stable for six months from the date of purchase provided that the components are stored appropriately. Store the 96-well plate, HRP conjugate stock and IgG stock vials at or below -20°C. Store the remaining components in the refrigerator at 2-8°C. The microtiter plate should always 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 except the HRP stock 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.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

DILUENT PREPARATION

The dilution buffer is provided as a 10x stock. Determine the volume of diluent required for your assay and dilute 1 volume of 10x diluent with 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. Reconstitute the goat IgG stock as detailed on the vial label. Vortex or mix to ensure complete reconstitution. *The reconstituted standard is stable at 2-8°C for one day*.
2. Label 6 polypropylene or glass tubes as 250, 125, 62.5, 31.25, 15.63 and 7.81 ng/ml.
3. Into the tube labeled 250 ng/ml, pipette the volume of diluent detailed on the IgG stock vial label. Then add the indicated volume of IgG stock and mix gently. This provides the 250 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labelled 125, 62.5, 31.25, 15.63 and 7.81 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 serial dilution.

SAMPLE PREPARATION

We found that IgG is present in normal goat serum at concentrations of ~10 mg/ml. In order to obtain values within range of the standard curve we suggest that samples initially be diluted 150,000-fold using the following procedure.

1. Dispense 998 µl and 897 µl of 1x diluent into two tubes.
2. Pipette and mix 2.0 µl of the sample into the first tube containing 998 µl of diluent. This provides a 500-fold dilution.
3. Mix 3.0 µl of the 500-fold diluted sample with the 897 µl of diluent in the second tube. This provides a 150,000-fold dilution of the sample.

HRP CONJUGATE PREPARATION

The HRP conjugate should be prepared approximately five minutes before required. The HRP conjugate stock should be diluted with diluent as detailed on the stock vial label.

PROCEDURE

1. Secure the desired number of 8-well strips in the cassette. Store unused strips at -20°C in a sealed plastic bag with desiccant.
2. Dispense 100 µl of standards and diluted samples into the wells (we recommend that standards and samples be run in duplicate).

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3. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C[1] 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 residual wash buffer.
6. Add 100 µl of diluted HRP conjugate into each well.
7. Incubate on an orbital micro-plate shaker at 150 rpm at 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. Incubate on an orbital micro-plate shaker at 150 rpm at 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*.

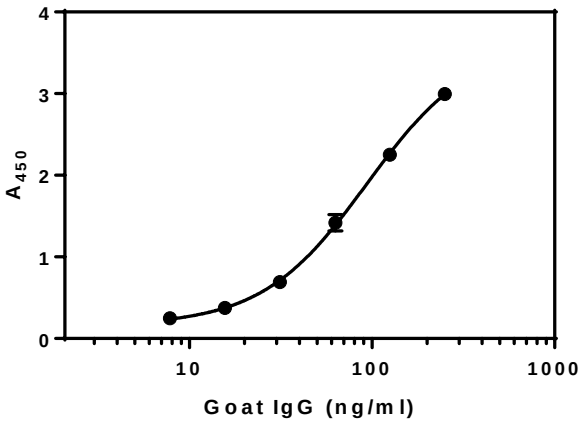
CALCULATION OF RESULTS

1. Using graphing software, construct a standard curve by plotting the absorbance values for the standards versus the $\log_{10}$ of the IgG concentration and fit the data to a four-parameter logistic equation.
2. Derive the corresponding concentration of IgG 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 IgG 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 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. A standard curve should be generated in each experiment.

IgG (ng/ml)	A_{450}
250	2.995
125	2.250
32.5	1.418
31.25	0.693
15.63	0.374
7.81	0.247



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

[1]The ELISA was validated using a shaking incubator at 150 rpm and 25°C. Operation of the assay at lower temperatures and mixing speeds will likely give lower absorbance values.