Sheep IgG ELISA

INTRODUCTION

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

PRINCIPLE OF THE ASSAY

Samples are diluted and incubated in the microtiter wells for 45 minutes alongside sheep 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. 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 sheep IgG coated 96-well plate **Store ≤ -20°C**
- HRP conjugate stock, 50 µl **Store ≤ -20°C**
- Sheep IgG stock (lyophilized), 3 vials **Store ≤ -20°C**
- 20x Wash solution: TBS50-20, 50 ml
- 10x Diluent: CSD25-10, 25 ml
- TMB reagent: 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
- Plate washer
- Plate reader with an OD range of 0-4 at 450 nm
- PC graphing software or graph paper

STORAGE

The test 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 4°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.

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 sheep IgG stock as detailed on the vial label. Vortex or mix to ensure complete reconstitution. ***The reconstituted standard is stable at 4°C for one day***.
2. Label 6 polypropylene or glass tubes as 250, 125, 62.5, 31.25, 15.63 and 0 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 0 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 62.5, 31.25 and 15.63 ng/ml standards by serial dilution.

SAMPLE PREPARATION

We found that IgG is present in normal sheep serum at concentrations of approximately 25 mg/ml. To obtain values within range of the standard curve we suggest that samples initially be diluted 600,000-fold using the following procedure.

1. For each sample to be tested dispense 198 µl into two separate tubes and 295 µl of 1x diluent into a third tube.
2. Pipette and mix 2.0 µl of the sample into the first tube containing 198 µl of diluent. This provides a 100-fold dilution.
3. Pipette and mix 2.0 µl of the 100-fold diluted sample into the second tube containing 198 µl of diluent. This provides a 10,000-fold dilution.
4. Mix 5.0 µl of the 10,000-fold diluted sample with the 295 µl of diluent in the third tube. This provides a 600,000-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.

ASSAY 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).
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*.

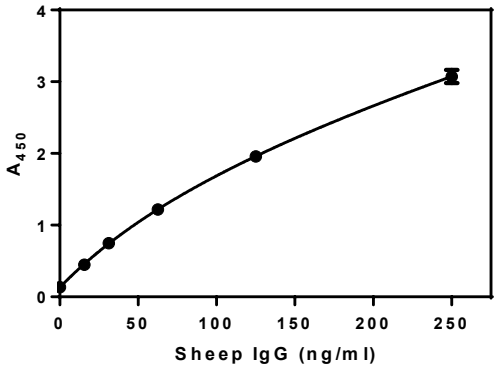
CALCULATION OF RESULTS

1. 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 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 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 should be used for the above steps if available. We recommend a fit using a second order polynomial equation or a single site, total and nonspecific binding equation.
6. 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 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	3.073
125	1.959
32.5	1.220
31.25	0.749
15.63	0.449
0	0.135



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
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.