



User's Manual

Sheep IgA ELISA Kit



DEIA-BY046



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This kit can be used for quantitative measurement of IgA in sheep serum or plasma.

Principles of Testing

Samples are diluted and incubated in the microtiter wells for 45 minutes alongside sheep 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. 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 IgA is proportional to the optical density of the test sample and is derived from a standard curve.

Reagents And Materials Provided

1. Anti sheep IgA coated 96-well plate
2. HRP conjugate stock, 50 ml
3. Sheep IgA stock (lyophilized), 3 vials
4. 20x Wash solution, 50 ml
5. Diluent, 50 ml
6. TMB reagent, 11 ml
7. Stop solution (1N HCl), 11 ml

Materials Required But Not Supplied

1. Precision pipettes and tips
2. Distilled or deionized water
3. Polypropylene or glass tubes
4. Vortex mixer
5. Absorbent paper or paper towels
6. Micro-plate incubator/shaker
7. Plate washer
8. Plate reader with an OD range of 0-4 at 450 nm
9. 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, IgA stock and HRP conjugate 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.

Specimen Collection And Preparation

We found that IgA is present in normal sheep serum at a concentration of approximately 4 mg/ml. In order to obtain values within range of the standard curve we suggest that samples initially be diluted 2000 fold. This can be accomplished using the following procedure for each sample to be tested.

1. Dispense 195 ml and 245 ml of 1x diluent into separate micro centrifuge tubes.
2. Add 5 ml of serum or plasma to the tube containing 195 ml of 1x diluent and gently mix. This provides a 40-fold dilution of the sample.
3. Add 5 ml of the 40-fold diluted sample to the tube containing 245 ml of 1x diluent and gently mix. This provides a 2000-fold dilution of the sample.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (25°C) before use.
2. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.
3. 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.
4. HRP Conjugate Preparation: The HRP conjugate should be prepared approximately five minutes before required. Dilute the HRP conjugate stock with diluent as detailed on the stock vial label.
5. Standard Preparation:
 - 1) Reconstitute the sheep IgA stock as detailed on the vial label. Vortex or mix to ensure complete reconstitution. The reconstituted standard is stable at 2-8°C for at least one day but should be aliquoted and frozen at -20°C after reconstitution if future use is intended.
 - 2) Label 6 polypropylene or glass tubes as 5.0, 2.5, 1.25, 0.625, 0.313 and 0.156 mg/ml.
 - 3) Into the tube labeled 5.0 mg/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 5.0 mg/ml standard.
 - 4) Dispense 250 ml of diluent into the tubes labelled 2.5, 1.25, 0.625, 0.313 and 0.156 mg/ml.
 - 5) Prepare a 2.5 mg/ml standard by diluting and mixing 250 ml of the 5.0 mg/ml standard with 250 ml of diluent in the tube labeled 2.5 mg/ml.
 - 6) Similarly prepare the remaining standards by serial dilution.

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 ml of standards and diluted samples into the wells (we recommend that standards and samples be tested in duplicate).



3. Incubate on an orbital micro-plate shaker at 150 rpm at 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 ml/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

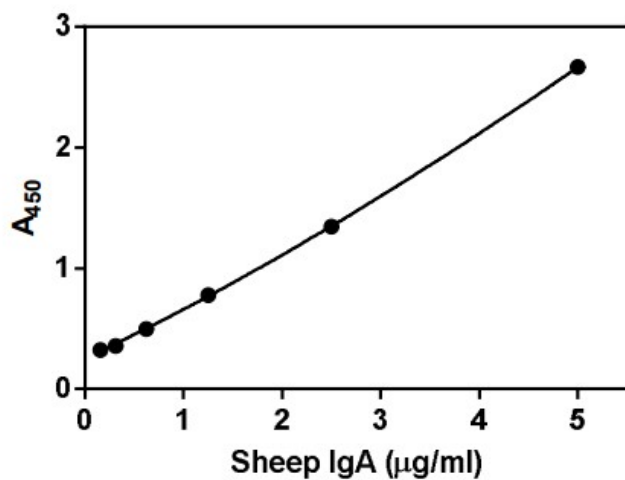
1. Calculate the average absorbance values (A₄₅₀) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgA from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgA 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 A₄₅₀ 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 IgA 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.



IgA ($\mu\text{g/ml}$)	A_{450}
5.0	2.698
2.5	1.371
1.25	0.792
0.625	0.542
0.313	0.378
0.156	0.361



Precautions

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.

