

Goat IgA ELISA

Life Diagnostics, Inc., Catalog Number: IGA-13

INTRODUCTION

This ELISA kit is designed for measurement of IgA in goat serum and plasma¹. The assay uses rabbit anti-goat IgA for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated rabbit anti-goat IgA for detection. Both capture and detection antibodies react specifically with IgA. 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 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.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-goat IgA coated 96-well plate **Store ≤ -20°C**
- HRP conjugate stock, 50 µl **Store ≤ -20°C**
- Goat IgA 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: SS-11, 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 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 4°C. The microtiter plate should always be kept in a sealed bag with desiccant.

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 diluent is provided as a 10x stock. Prior to use estimate the final volume of 1x 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. Reconstitute the goat IgA 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 but should be aliquoted and frozen at -20°C after reconstitution if future use is intended.**
2. Label 6 polypropylene or glass tubes as 250, 125, 62.5, 31.25, 15.63 and 7.81ng/ml.
3. Into the tube labeled 250 ng/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 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 IgA is present in normal goat serum at concentrations of ≈100 µg/ml. 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 µl and 245 µl of 1x diluent into separate micro centrifuge tubes.
2. Add 5 µl of serum or plasma to the tube containing 195 µl of 1x diluent and gently mix. This provides a 40-fold dilution of the sample.
3. Add 5 µl of the 40-fold diluted sample to the tube containing 245 µl of 1x diluent and gently mix. This provides a 2000-fold dilution of the sample.

¹ Although we have not tested goat milk in this ELISA it is expected that the assay will detect IgA in milk. Optimum dilutions should be determined empirically.

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 tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 45 minutes.²
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.

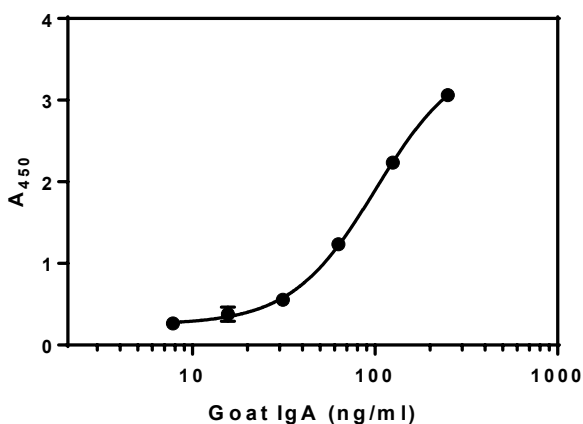
CALCULATION OF RESULTS

1. Using graphing software, construct a standard curve by plotting the absorbance values for the standards versus the log₁₀ of the IgA concentration and fit the data to a four-parameter logistic equation.
2. Derive the corresponding concentration of IgA 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 IgA 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 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 (ng/ml)	A ₄₅₀
250	2.614
125	1.688
62.5	0.877
31.25	0.393
15.63	0.147
7.81	0.139



Rev 11182020

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