Goat IgM ELISA

INTRODUCTION

This ELISA kit is designed for measurement of IgM in goat serum and plasma. The assay uses rabbit anti-goat IgM for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated rabbit anti-goat IgM for detection. Both capture and detection antibodies react specifically with goat IgM. 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 IgM standards. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. IgM 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 IgM 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 IgM coated 96-well plate (12 x 8-well strips) **Store ≤ -20°C**
- HRP conjugate stock, 50 µl **Store ≤ -20°C**
- Goat IgM 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: 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 IgM 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 goat IgM 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 5 polypropylene or glass tubes as 200, 100, 50, 25 and 12.5 ng/ml.
3. In the tube labeled 200 ng/ml, pipette the volume of diluent detailed on the IgM stock vial label. Then add the indicated volume of IgM stock and mix gently. This provides the 200 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labelled 100, 50, 25, and 12.5 ng/ml.
5. Prepare a 100 ng/ml standard by diluting and mixing 250 µl of the 200 ng/ml standard with 250 µl of diluent in the tube labeled 100 ng/ml.
6. Similarly prepare the remaining standards by serial dilution.

SAMPLE PREPARATION

We found that IgM is present in normal goat serum at concentrations of approximately 2.5 mg/ml. To obtain values within range of the standard curve we suggest that samples initially be diluted 25,000-fold using the following procedure.

1. Dispense 498 µl and 495 µl of 1x diluent into two tubes.
2. Pipette and mix 2.0 µl of the sample into the first tube containing 498 µl of diluent. This provides a 250-fold dilution.
3. Mix 5.0 µl of the 250-fold diluted sample with the 495 µl of diluent in the second tube. This provides a 25,000-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.

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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*.

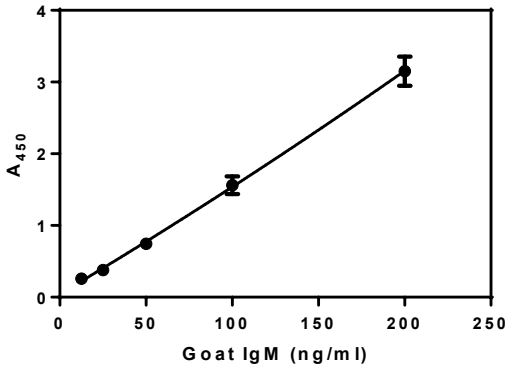
CALCULATION OF RESULTS

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 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 IgM in ng/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgM 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.
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 IgM 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.

IgM (ng/ml)	A_{450}
200	3.152
100	1.563
50	0.746
25	0.380
12.5	0.257



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.