Rabbit IgM ELISA
Life Diagnostics, Inc., Catalog Number: IGM-10

Rabbit IgM ELISA

INTRODUCTION

The Rabbit IgM ELISA kit is intended for measurement of IgM in serum and plasma. Goat anti-rabbit IgM heavy chain antibodies are used for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated goat anti-rabbit IgM heavy chain antibodies are used for detection. Normal rabbit IgM levels are in the range of 0.2-3.0 mg/ml in serum or plasma.

PRINCIPLE OF THE TEST

Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside prepared Rabbit 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. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. 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 spectrophotometrically 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 Rabbit IgM coated 96-well plate (12 strips of 8 wells)
- HRP Conjugate Reagent, 11 ml
- Reference standard (lyophilized)[1]
- 20x Wash Solution: TBS50-20, 50 ml
- 10x Immunoglobulin Diluent: RD25-10, 25 ml
- TMB Reagent (One-Step): 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 mixing speed of 150 rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450nm
- Graph paper (PC graphing software is optional)

STORAGE

The unused kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents 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 diluent is provided as a 10x stock. Prior to use estimate the final volume of 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. The IgM standard is provided as a lyophilized stock. Reconstitute with 1.0 ml of distilled or deionized water **(the reconstituted standard is stable at 4°C for at least one day but should be aliquoted and frozen at -20°C after reconstitution if future use is intended)**.
2. Label 8 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 IgM standard vial label. Then add the indicated volume of IgM standard and mix gently. This provides the 250 ng/ml standard.
4. Dispense 250 μl of diluent into the tubes labeled 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 62.5, 31.25, 15.63 and 7.81 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: To obtain values within range of the standard curve, we suggest that samples initially be diluted 25,000-fold using the following procedure for each sample to be tested:

1. Pipette 5.0 μl of the serum/plasma sample into a tube containing 495 μl diluent and mix. This provides a 100-fold diluted sample.
2. Mix 2.0 μl of the 100-fold diluted sample with the 498μl of diluent in a second tube. This provides a 25,000-fold dilution of the sample.
3. Repeat this procedure for each sample to be tested.

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[1]The reference standard was calibrated with purified Rabbit IgM

PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μl of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (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 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 all residual wash buffer.
6. Add 100 μl of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 μl of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (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 concentrations by the dilution factor to determine the actual concentration of IgM in the sample.
5. PC graphing software may be used for the above steps.
6. If the OD_{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 450nm 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. Each user should obtain his or her data and standard curve in each experiment.

IgM (ng/ml)	Absorbance (450 nm)
250	3.232
125	2.232
62.5	1.262
31.25	0.666
15.63	0.314
7.81	0.218
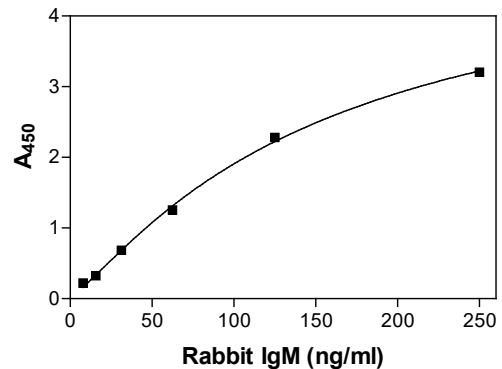
LIMITATIONS OF THE PROCEDURE

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
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