EAGLE BIOSCIENCES

Rabbit IgM ELISA

Catalog Number:
IGM79-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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Introduction

The Eagle Biosciences Rabbit IgM ELISA Assay Kit is designed for the quantitative determination of rabbit IgM in complex samples (serum or other biological samples). The Rabbit IgM ELISA Assay Kit is for research use only and should not be used in diagnostic procedures.

Principle of the Assay

The determination of rabbit IgM is carried out as direct sandwich ELISA. An antibody specific for rabbit IgM has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgM present is bound. After washing away any unbound substances, an enzyme-linked antibody is added. Following a wash, a substrate solution is added to the wells and color develops in proportion to the amount of antibody conjugate. The absorption at 450 nm is proportional to the IgM concentration.

Precautions

- Store the Rabbit IgM ELISA Assay Kit at 2-8 °C.
- The Rabbit IgM ELISA Assay Kit is for research use only. Not for use in diagnostic procedures.
- Do not use the reagents beyond the expiration date marked on box label.
- Please read the instructions carefully before using the Rabbit IgM ELISA Assay Kit.
- Do not mix reagents from different lots.
- Some components of this Rabbit IgM ELISA Assay Kit contain Thimerosal, a mercury containing compound. The stop solution contains 0.5 M sulphuric acid. Follow routine precautions for handling hazardous chemicals.

Other supplies required

- Deionized or distilled water
- Graduated cylinder
- Micropipettes, multipipette
- Microplate reader

Preparation of reagents and samples

- Bring all reagents of the Rabbit IgM ELISA Assay kit to room temperature before use. If crystals have formed, mix gently until the crystals have completely dissolved.

- The microplate strips are ready to use. Remove excess strips (breakable) from the frame, reseal in the bag with the desiccant and store at 2-8 °C

- Dilute the wash buffer with deionized or distilled water **1:10** (e. g. 40 ml + 360 ml water). The diluted solution is stable for 30 days at 2-8 °C.

- Use the Standard concentrate to produce a 1:2-dilution series with diluent (e. g. 250 µl + 250 µl diluent):

Standard	Preparation	Conc. (ng/ml)
S 7	standard conc. Undiluted	1000
S 6	S 7 1:2 diluted	500
S 5	S 6 1:2 diluted	250
S 4	S 5 1:2 diluted	125
S 3	S 4 1:2 diluted	62.5
S 2	S 3 1:2 diluted	31.25
S 1	S 2 1:2 diluted	15.625

- Dilute the samples with diluent. If samples generate values outside the standard curve, the dilution factor may be varied. To exclude matrix effects the dilution factor should be at least 1:50.

Assay procedure

It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, standard curve and samples as directed in the previous section.
2. Pipette 100 µl of samples, standards, positive control or diluent (as negative control) into the wells.
3. Seal wells with adhesive strip and incubate for 1 hour at room temperature with shaking *.
4. Aspirate fluid from wells and wash three times with 300 µl wash buffer. After the last wash, invert the plate and tap on a clean paper towel.
5. Add 100 µl of HRP conjugate to each well.
6. Seal wells with adhesive strip and incubate for 1 hour at room temperature with shaking *.
7. Repeat the wash as in step 4.
8. Dispense 100 µl of TMB substrate solution into each well.
9. Incubate for 10 minutes at room temperature in the dark.
10. Add 100 µl of stop solution to each well.
11. Determine the absorbance within 30 minutes at 450 nm. A reference wavelength of 620 nm/690 nm is recommended.

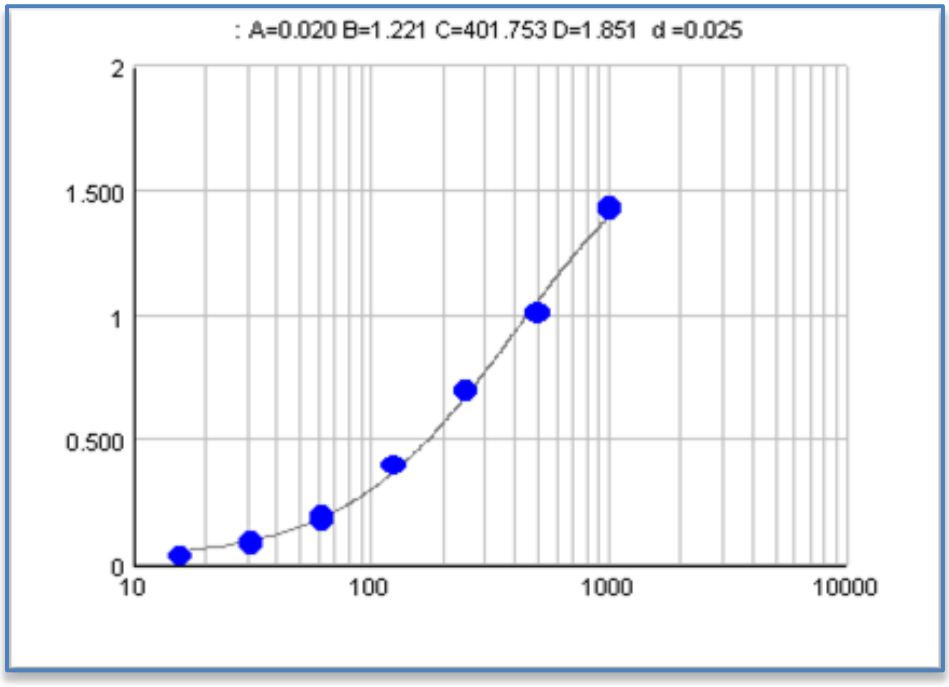
*: If a MTP shaker is not available, it is possible to incubate for 2 hours without shaking.

Calculation of results

Create a standard curve for the Rabbit IgM ELISA Assay Kit using computer software capable of generating a curve fit (4 parameter fit; x-axis: log, IgM concentration; y-axis: linear, absorbance). As an alternative, draw a standard curve on semi-log paper (x-axis: log, IgM concentration; y-axis: linear, absorbance). The IgM concentrations can be calculated from the standard curve. The calculated concentrations must be multiplied by the sample dilution factor.

If the absorbance of some samples is outside the standard curve a subsequent determination with changed dilutions will provide a proper result.

Typical Standard Curve



Materials provided:

Number of determinations	1x96 determinations
Microplate strips, antibody coated	12 x 8
Wash buffer, 10fold conc. ✦	50 ml
Diluent, ready to use ✦	100 ml
Standard concentrate, 1000 ng/ml ✦	2 ml
Anti-IgM(rabbit)-Ab., HRP conjugate, ready to use	12 ml
TMB substrate, ready to use	12 ml
Stop solution, ready to use (0.5 M sulphuric acid)	12 ml

✦: contains Thimerosal

Performance Characteristics

- *Standard curve:*
  7 standards between 15.625 ng/ml and 1000 ng/ml

- *Sensitivity:*
  782 ng/ml (sample dilution 1:50)
- *Sample materials:*
  Protein solutions, serum, body fluids
- *Intraassay precision (CV):*
  (n=10)
  at 42.0 ng/ml: 3.1%
  at 176.9 ng/ml: 2.3%
  at 382.1 ng/ml: 5.5%

A. Preparation
1. Bring all reagents to room temperature
2. Dilute wash buffer 1:10
3. Prepare the standard curve from a 1:2-dilution series of standard concentrate with diluent
4. Dilute samples with diluent

B. Performance
1. Pipette 100 µl of samples, standards, controls into the wells
2. Incubate for 1 hour at room temperature with shaking
3. Wash three times with 300 µl of wash buffer
4. Add 100 µl of HRP conjugate to each well
5. Incubate for 1 hour at room temperature with shaking
6. Wash three times with 300 µl of wash buffer
7. Dispense 100 µl of TMB substrate solution
8. Incubate for 10 minutes at room temperature in the dark
9. Add 100 µl of stop solution
10. Measure absorption at 450 nm

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.