Rat IgM ELISA

Catalog Number:
IGM29-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 Rat IgM ELISA Assay Kit is designed for the quantitative determination of rat IgM in complex samples (serum or other biological samples). The Rat IgM ELISA Assay Kit is for research use only and should not be used in diagnostic procedures.

Principle of the Assay

The determination of rat IgM is carried out as direct sandwich ELISA. An antibody specific for rat 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 Rat IgM ELISA Assay Kit at 2-8 °C.
- The Rat 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 Rat IgM ELISA Assay Kit.
- Do not mix reagents from different lots.
- Some components of this Rat 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 Rat 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 plastic bag with the desiccant and store at 2-8 °C

- Dilute the wash buffer with deionized or distilled water **1:10** (e. g. 50 ml + 450 ml water). The diluted solution is stable for 30 days at 2-8 °C.

- Dilute the HRP conjugate with diluent **1:100** (e. g. 50 µl + 4950 µl diluent). The required amount of conjugate solution should be prepared freshly.

- 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	2000
S 6	S 7 1:2 diluted	1000
S 5	S 6 1:2 diluted	500
S 4	S 5 1:2 diluted	250
S 3	S 4 1:2 diluted	125
S 2	S 3 1:2 diluted	62.5
S 1	S 2 1:2 diluted	31.25

Dilute the samples with diluent. To exclude matrix effects the dilution factor should be at least 1:10. If samples generate values outside the standard curve, the dilution factor may be varied.

- Normal rat serum contains about 4 mg IgM/ml.

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, or diluent (as negative control) into the wells.
3. Seal wells with adhesive strip and incubate for 1 hour at room temperature with shaking (recommended) or 2 hours without 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 (recommended) or 2 hours without shaking.
7. Repeat the wash as in step 4.
8. Dispense 100 µl of TMB substrate solution into each well.
9. Incubate for 20 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.

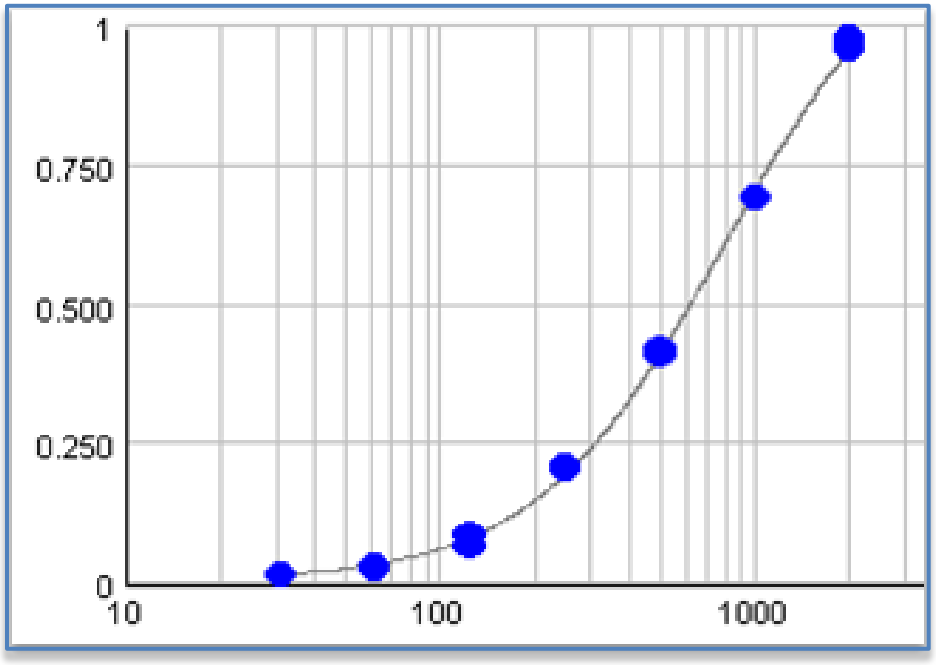
Calculation of results

Create a standard curve for the Rat 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 samples 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, 2000 ng/ml ✦	2 ml
Anti-IgM(rat)-Ab., HRP conjugate, 100fold conc. ✦	0,2 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 31.25 ng/ml and 2000 ng/ml
- *Sensitivity:*
  312.5 ng/ml (sample dilution 1:10)
- *Sample materials:*
  Protein solutions
- *Intraassay precision (CV):*
  (n=10)
  at 70 ng/ml: 7.6%
  at 250 ng/ml: 6.8%

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
5. Dilute freshly HRP conjugate 1:100 with diluent

B. Performance

1. Pipette 100 µl of samples, standards, diluent (blank) into the wells
2. Incubate for 1 hour at room temperature with shaking (or 2 hrs without 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 (or 2 hrs without shaking)
6. Wash three times with 300 µl of wash buffer
7. Dispense 100 µl of TMB substrate solution
8. Incubate for 20 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.