Molecular Innovations®
www.mol-innov.com

Rat IgM Antigen ELISA Kit

Catalog # RTIGMKT

Strip well format. Reagents for up to 96 tests.

Rev: August 2015

INTENDED USE

This Rat Immunoglobulin M (IgM) antigen assay is intended for the quantitative determination of total rat IgM antigen in serum, plasma, hybridoma cell supernatants, ascites or other biological fluids.

For research use only.

BACKGROUND

IgM is the first immunoglobulin produced in the immune response and is the third most abundant immunoglobulin in serum. IgM is a disulfide-linked 970kDa pentamer that activates complement and is responsible for red blood cell agglutination. Each monomer consists of two mu heavy chains and two kappa or lambda light chains.

ASSAY PRINCIPLE

Rat IgM will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, horseradish peroxidase labeled polyclonal anti-rat IgM antibody binds to the captured protein. Excess antibody is washed away and TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of rat IgM. Color development is directly proportional to the concentration of total IgM in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-rat IgM antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Rat IgM standard:** 1 vial lyophilized standard
- **Anti-rat horseradish peroxidase antibody:** 1 vial lyophilized polyclonal antibody
- **TMB substrate solution:** 1 bottle of 10ml

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard may be stored at -80°C for later use. Do not freeze-thaw the standard more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1,000ng/ml standard solution.

Dilution table for preparation of rat IgM standard:

IgM concentration (ng/ml)	Dilutions
500	500µl (BB) + 500µl (from std vial)
250	500µl (BB) + 500µl (500ng/ml)
100	600µl (BB) + 400µl (250ng/ml)
50	500µl (BB) + 500µl (100ng/ml)
25	500µl (BB) + 500µl (50ng/ml)
10	600µl (BB) + 400µl (25ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2.5	500µl (BB) + 500µl (5ng/ml)
1	600µl (BB) + 400µl (2.5ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl IgM standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures IgM antigen in the 0.5-500 ng/ml range. If the unknown is thought to have high IgM levels, dilutions may be made in blocking buffer. A 1:1,000 to 1:10,000 dilution for normal rat plasma or serum is suggested for best results.

Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

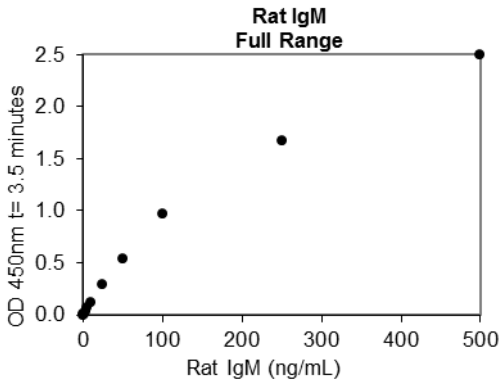
Measurement

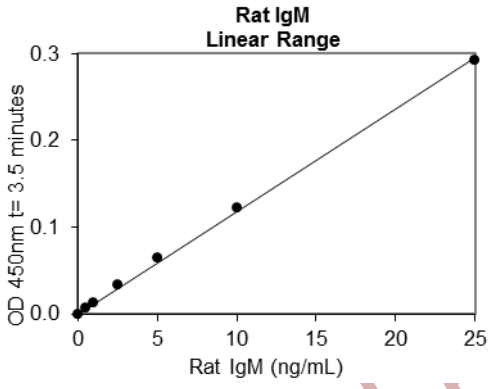
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of IgM in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of IgM in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):





EXPECTED VALUES

The concentration of IgM in normal rat serum ranges from 0.2 to 0.5 mg/mL [1].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD450: 0.041-0.045) and calculating the corresponding concentration. The MDD was 0.25 ng/ml.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: This assay recognizes total rat IgM. Pooled normal plasma from human, mouse, rabbit, rhesus monkey, horse, dog, sheep and pig was assayed and no significant cross-reactivity was observed.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (µg/mL)
Citrate Plasma	1:5,000	58.9
	1:10,000	60.2
	1:20,000	64.4

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Salauze D. *et al.*: Comp Haematol Int. 1994, 4(1):30-3.

Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	
B	0	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	
C												
D												
E												
F												
G												
H												