PERFORMANCE CHARACTERISTICS (continued)

Sample Recovery
High and low concentrations of rat IgM were mixed into each of 3 serum samples. Observed assay values compared to expected values ranged from 97 to 114%, indicating accurate quantification of IgM in rat serum.

Sample	Expected ng/ml	Observed ng/ml	Observed/ Expected
High IgM Concn		56	
+ Rat serum C, 73.6 ng/ml	130	134	**97**
+ Rat serum D, 42 ng/ml	98	100	**98**
+ Rat serum F, 51 ng/ml	107	107	**100**
Low IgM Concn		28	
+ Rat serum C, 73.6 ng/ml	102	100	**99**
+ Rat serum D, 42 ng/ml	70	70	**100**
+ Rat serum F, 51 ng/ml	79	79	**100**

Related Items

Catalog#	Product Description
6410	Rat serum amyloid P (SAP) ELISA Kit, 96 tests, Quantitative
6410-10	Rat IgA ELISA kit 96 tests, Quantitative
6420	Rat IgG ELISA Kit, 96 tests, Quantitative
6420-RDT-25	TruStrip RDT Rat IgG Rapid Test cards, 10/pk
6430	Rat IgG1 ELISA Kit, 96 tests, Quantitative
6440	Rat IgG2a ELISA Kit, 96 tests, Quantitative
6450	Rat IgG2b ELISA Kit, 96 tests, Quantitative
6470	Rat IgE ELISA Kit, 96 tests, Quantitative
6480	Rat IgM ELISA Kit, 96 tests, Quantitative
6490	Rat Alpha-1 Glycoprotein (A1-AGP) ELISA kit 96 tests, Quantitative

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. M-6480

Rat IgM ELISA Kit

Cat. No. 6480, 96 tests

For Quantitative Determination of Rat IgM in Serum, plasma, or other biological fluids

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

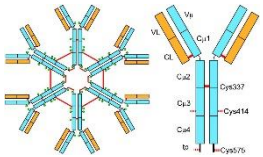
ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777
Email: service@4adi.com
Web Site: www.4adi.com

INTENDED USE

The Rat IgM ELISA Kit is an in vitro immunoassay for research use in the quantification of Rat IgM circulating in serum or in other appropriately qualified samples from tissue fluids (e.g., saliva, mucosa), or in cultures of rat cells. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

RESEARCH USE OF THE TEST

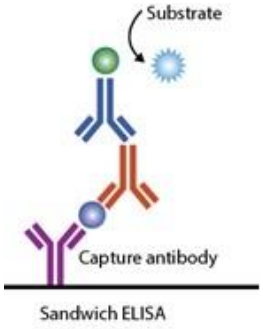


Immunoglobulin M (IgM) is a basic antibody that is produced by B cells. IgM is by far the physically largest antibody in the human circulatory system. It is the first antibody to appear in response to initial exposure to an antigen. IgM is produced and secreted from spleen, a major site for B cell activity. IgM forms polymers where multiple immunoglobulins are covalently linked together with disulfide bonds, mostly as a pentamer (970 kda) but also as a hexamer. Because each monomer has two antigen binding sites, a pentameric IgM has 10 binding sites. The J chain is found in pentameric IgM but not in the hexameric form. Due to its polymeric nature, IgM possesses high avidity, and is particularly effective at complement activation. It contributes greatly to opsonization by activating complement and causing C3b to bind to the antigen. IgM normal plasma concentration ~1.-4 mg/ml. Low levels of IgM are associated with Wiskott-Aldrich syndrome.

Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of rat immunoglobulins IgG, IgA and IgM, as found circulating in blood or as present in other body fluids, including saliva, milk/colostrum, ascites, tears and mucosa of linings of the gut, respiratory or urogenital tracts. Levels of total IgG, IgA and/or IgM can reveal health status or results of experimental or pathological conditions (e.g., hypo- or hyper-gammaglobulinemia or acute or chronic infection). Also, measurements of specific antibody levels, in antigen-specific assays, are often best interpreted relative to values of total IgG, IgA, and IgM in the sample and/or individual.

The quantitative immunoassay measures rat IgM with high sensitivity; this allows dilution beyond interference from the sample matrix for samples derived from any of the above specimen types. Expected performance relative to precision, recovery and linearity of dilution is presented for guidance of use and experimental design.

PRINCIPLE OF THE TEST



The Rat IgM ELISA kit is based on the binding of rat IgM in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of IgM present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of IgM in samples and control is calculated from a curve of standards containing known concentrations of IgM.

PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

Specificity

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with IgM, and have essentially no reactivity with IgG, IgA, IgE or any other rat serum proteins.

Serum from mouse and hamster showed some reactivity, and the following species showed no significant reactivity at 1:400 dilution: human, monkey, guinea pig, chimp, pig, horse, sheep, goat, dog, cat, rabbit or chicken; also 10% neonatal bovine serum.

Normal Range

Assay of IgM in stored sera from ten (10) individual adult rats ranged from 0.36 to 1.55 mg/ml (median = 0.88 mg/ml). Each laboratory should determine expected values of its own testing population.

Precision

Samples containing low, medium and high concentrations of IgM were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation (CVs) were calculated for the concentrations using a point-to-point curve-fitting program.

IgM concentrations were measured with good within-assay (4.6 to 5.7 %CV) and between-assay (3.1 to 6.1 %CV) reproducibility.

Sample	IgM ng/ml	Intra-assay %CV	Inter-assay %CV
Low Sample	35	**5.1**	**4.3**
Mid Sample	93	**5.7**	**3.1**
High Sample	147	**4.6**	**6.1**

Linearity of Dilution

Three (3) individual stored sera were diluted to 2 levels for testing, and concordance of the assay values was compared. Agreement of values ranged from 94 to 97%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Rat Serum 1	1:10k 1:80k	127 17	1.27 1.36	**97 %**
Rat Serum 2	1:5k 1:40k	153 20	0.76 0.80	**97 %**
Rat Serum 3	1:5k 1:40k	135 19	0.68 0.76	**94 %**

CALCULATIONS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Rat IgM concentrations may be determined as follows:

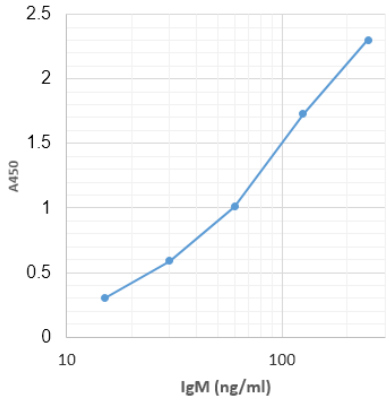
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of Rat IgM (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The Rat IgM concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 250 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	Rat IgM ng/ml
1A, B	**Negative Diluent Control**	0.02	0
1C, D	15 ng/ml **Standard**	0.30	15
1E, F	30 ng/ml **Standard**	0.59	30
1G, H	60 ng/ml **Standard**	1.01	60
2A, B	125 ng/ml **Standard**	1.73	125
2C, D	250 ng/ml **Standard**	2.30	250
2E, F	**Positive Serum Control** [Value: 70 - 130 ng/ml]	1.41	91
2G, H	**Sample** [Diluted 1:10k]	1.20	73
	Calculated: 10k-fold dilution x 73 ng/ml = **0.73** mg/ml in serum		

A typical assay Standard Curve (do not use for calculating sample values)



B/6480-Rat-IgM-ELISA-Graph

KIT CONTENTS

Ready For Use: Store as indicated on labels.

Component	Part #	Amt	Contents
Anti-Rat IgM Microwell Strip Plate	6481	8-well strips (12)	Coated with purified anti- Rat IgM antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
Positive Control [IgM] range on label	6482	0.65 ml	Rat serum with stated IgM concentration range; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
Rat IgM Standards			
15 ng/ml	6483B	0.65 ml	Five (5) vials, each containing rat serum calibrated using purified rat IgM; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
30 ng/ml	6483C	0.65 ml	
60 ng/ml	6483D	0.65 ml	
125 ng/ml	6483E	0.65 ml	
250 ng/ml	6483F	0.65 ml	
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume, 10ml, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Rat IgM-HRP Conjugate Concentrate (100x) Part No. 6484, 0.15ml	Peroxidase conjugated anti-rat IgM antibody in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8ºC storage.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the kit label. Stabilities of the working solutions are indicated under Reagent Preparation.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent Concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and Antibody-HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site for Prolcin-300 (0.1% v/v in standards, and assay buffers).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference.

For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For **other samples**, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately , store refrigerated for up to a week, or frozen for long-term storage. Avoid freeze-thaw cycles.

QUALITY CONTROL

Sample Controls A Positive Serum Control is provided with the kit, assigned with an IgM concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer is recommended. ELISA reader and pipettes should be properly calibrated.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30^{o} C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 1:10k-1:20k are appropriate for most normal rat sera. For accuracy, three dilution steps are recommended, as follows:

1) 10ul serum + 990ul diluent = [1:100],
2) 10ul [1:100] + 990ul diluent = **[1:10k].**

DO NOT dilute the Standards or Positive Control Serum.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, to include 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand about 5 minutes before sample addition.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of Working Anti-Rat IgM-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.