ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Folicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#**0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (toal)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-3000

Rheumatoid Factor IgM (RF IgM)

ELISA KIT Cat. No. 3000, 96 Tests

For Quantitative Determination of RF IgM In Human Serum

For In Vitro Research Use Only

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Rheumatoid Factor IgM (RF IgM) ELISA KIT # 3000

For Quantitative Determination of RF IgM In Human Serum

Kit Contents: (reagents for 96 tests)

Components	
Purified gamma globulin coated microwell strips (96 wells), Ready-to-use, P-3001	1 Plate
RF IgM **Standard A** (0 IU/ml) 1.5 ml, #3002A	1 vial
RF IgM **Standard B** (15 IU/ml) 1.5 ml, #3002B	1 vial
RF IgM **Standard C** (50 IU/ml) 1.5 ml, #3002C	1 vial
RF IgM **Standard D** (150 IU/ml) 1.5 ml, #3002D	1 vial
RF IgM **Standard E** (500 IU/ml) 1.5 ml, #3002E	1 vial
RF IgM **Postive control**, 1.5 ml, #P3000	1 vial
RF IgM **Negative control**, 1.5 ml, #N3000	1 vial
RF IgM Sample Diluent (5X), 20 ml, #3003	1 bottle
Anti-hIgM HRP Conjugate, 15 ml, #3004	1 bottle
Wash buffer (50X), 20 ml, #WB-50 dilute 1:50 with distilled water,	1 bottle
HRP Substrate Solution, 15 ml, #TMB-3000	1 bottle
Stop solution, 15 ml, #ST-3000	1 bottle
Complete Instruction Manual	M-3000

Intended use:

ADI's Rheumatoid Factor IgM is for Quantitative Determination of RF IgM In Human Serum. For in-vitro research use only (RUO).

Introduction

The presence of IgM rheumatoid factor (RF) in the serum is the sole serological indicator included in the ACR list of criteria for the diagnosis of RA. RFs are a subset of antiglobulins directed against the Fc region of IgG. In this definition we do not include antibodies to the IgG Fab region and pepsin agglutinators, directed against neoantigens on IgG exposed by pepsin cleavage. It is claimed that the majority of antiglobulin activity in normal serum is Fab-specific, whereas an- tiglobulin from RA patients is mostly Fc-specific. RFs are present in the serum of 75-80% of patients with RA at some time during the disease course. However, RFs are also found in the serum of patients with infectious and autoimmune diseases, hyperglobulinemia, B-cell lymphoproliferative disorders and in the aged population. This suggests that RF may be a finding associated with B-cell hyperactivity.

Rheumatoid factors which have been found among the IgM, IgG and IgA classes of immunoglobulins, reacting only with xenogeneic Fc are not autoantibodies and are unlikely to be of pathological significance. However, RFs can bind IgG from many species, including autologous IgG, when immobilised on surfaces. Autologous binding is of higher affinity than xenogeneic binding. The here presented test systems for the determination of rheumatoid factors uses only human Fc fragments as coated antigen. It is generally considered that high RF titers are associated with more severe disease and the presence of extra-articular features and rheumatoid nodules. This conclusion may depend on the disease duration. Serum IgM RF may precede the onset of RA by several years. A high titer of RF in non-RA individuals is associated with increased risk of developing RA. In the first 2 years of RA (early RA), serum levels of IgM, IgG and IgA RF do not correlate with disease activity. Serum IgG and IgA RF in these years are prognostic of erosive joint disease. In established RA, high titer serum IgM RF correlates with the presence of articular disease and nodules but not with systemic disease activity. The presence of either IgG or IgA RF in patients with long-standing RA may be a good prognostic indicator of systemic manifestations. IgG and IgM RF are associated with extra-articular RA including rheumatoid vasculitis and nodules. The presence of IgM RF containing immune complexes with bound complement (C1q) is also associated with extra-articular RA.

PERFORMANCE CHARACTERISTICS

PRECISION

Intra-assay precision:

Sample	Mean (IU/ml)	**CV%**
1	29.2	2.4
2	116.3	3.6
3	294.4	4.9

Inter-assay precision:

Sample	Mean (IU/ml)	**CV%**
1	26.7	4.0
2	102.0	3.6
3	275.1	3.2

EXPECTED VALUES

It is recommended that each laboratory must determine it own negative and positive values. Samples containing less than 25 IU/ml RF IgM can be considered as RF IgM-negative; samples showing greater than 25 IU/ml concentrations can be considered as RF IgM-positive. Concentrations of higher than 75 IU/ml RF IgM usually indicate rheumatoid arthritis. RF IgM are found in about 2-10% of apparently healthy Caucasian adults and in about 50-70% of adults with classical rheumatoid arthritis.

Clinical Study

A clinical study using the ADI's RF IgM ELISA was conducted and results are summarized below:

	ADI	**Commercial ELISA**	**Commercial Latex test**
N	69	69	69
Negative Range	26 <25 IU/ml	29 <20 IU/ml	24 <1:20
Positive Range	15 25-75 IU/ml	14 20-60 IU/ml	15 1:20
Rheum. Arth. Range	28 >75 IU/ml	26 >60 IU/ml	30 >1:20

Species Crossrectivity

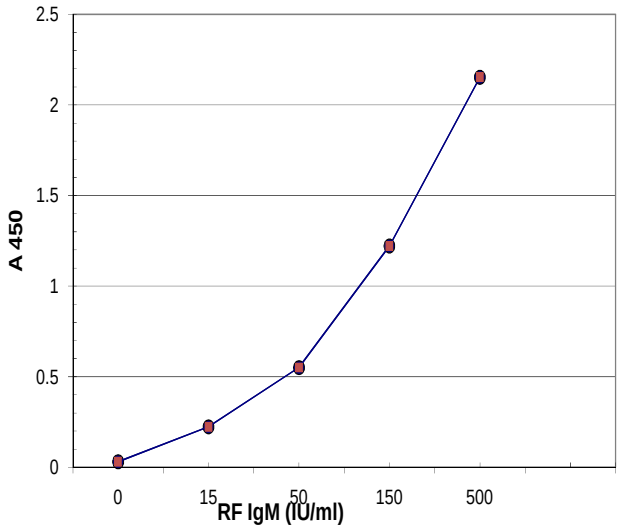
This kit is recommended for human samples only. Its utility in other species such as mouse, rat, or monkey etc has not been tested. ADI has a separate RF ELISA kit for mouse samples (#6200).

References: Johnson PM (1976) Clin. Immunol. 6, 414; Bartfield H (1969) Ann. NY Acad. Sci. 168, 30; Karah J (1980) J Immunol. Meth. 32, 115; Moors TL (1978) Arth Rheumat. 21, 935; Seymour P (1980) Am. Soc. Clin. Pathol. 74, 776; Puente A (1988) Arht. Rheumat. 31, 1230.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds (IU/ml)	Mean $A_{450\ nm}$	Calcul. Conc. (IU/ml)*
A1, A2	0	0.030	
B1, B2	15	0.223	
C1, C2	50	0.550	
D1, D2	150	1.222	
E1, E2	500	2.154	

NOTE: These data are for demonstration purpose only. A complete set of negative, positive, and calibrator standards set must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the sample diluents from the mean absorbance values of negative & positive controls, calibrator, and samples. The RF IgM values for the patient samples can be calculated as follows:

$$\text{IU/ml of sample} = \frac{\text{Net absorbance of test sample}}{\text{Net absorbance of the calibrator}} \times \text{IU/ml of calibrator}$$

PRINCIPLE OF THE TEST

Rheumatoid Factor IgM (RF IgM) ELISA kit is based on binding of RF IgM from serum samples to human gamma globulin immobilized on microtiter wells. After a washing step, anti-human IgM-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of RF IgM present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of RF IgM in samples is calculated on the basis of the absorbance of the negative, positive, and, calibrator controls.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Rheumatoid Factor IgM ELISA Kit is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Negative, Positive, and Calibrator controls have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed , these could be stored at -20^{o}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

REAGENTS PREPARATIONS:

Wash buffer is supplied as 50x stock. Dilute 20 ml into 980 ml de-ionized or distilled water, mix, and store at room temp for 1-2 weeks. It can be stored at 4oC for long term storage.
Sample Diluent (5X): **Dilute 20 ml into 80 ml de-ionized or distilled water.**

Dilute serum sample 1:100 in 1x sample diluent (5 ul sample in 495 ul buffer) .

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Label or mark the microtiter well strips to be used on the plate. Dilute serum samples 1:100 (5 µl of sample in a total volume of 500 µl of sample diluents). **Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter). Dilute Sample Diluent (5X)**: **Dilute 20 ml into 80 ml de-ionized or distilled water. Standards and controls are supplied pre-diluted.**

1. Pipet **100** «**l** of diluted sample diluents, negative & positive controls, calibrator, and diluted serum samples into appropriate wells in *duplicate*. Cover the plate and incubate for **30 minutes** at **room temperature** (20-28oC).

2. Aspirate and wash the wells **3 times** with 300 µl of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

3. Add **100** «**l** of antibody-enzyme conjugate into **each well.** Mix gently. Cover the plate and incubate for **15 minutes** at room temperature(20-28oC).

4. Aspirate and wash the wells **3 times** with 300 µl of diluted wash buffer, as above.

5. Dispense **100 ul TMB substrate per well**. Mix the plate gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temperature. Blue color develops into standards and positive samples.

8. Stop the reaction by adding **100** «**l of stopping solution** to **all wells** at the same timed intervals as in step 8. Mix gently. Blue color turns yellow.

9. Measure the absorbance at 450 nm using an ELISA reader.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

QUALITY CONTROL OF THE TEST

1. OD values will vary with the temperature and length of incubation.
2. The O.D. of the Standards A (reagents blank) should be <0.250 and Standard E >1.100.
3. The value of positive and negative controls should be within the range of indicated value.

Calculation of results

For Rheumatoid Factor IgM a 4-Parameter-Fit with lin-log or lin-lin coordinates for optical density and concentration is the data reduction method of choice.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Rheumatoid Factor IgM test:

	Rheumatoid Factor IgM [IU/ml]
Normal:	< 20
Elevated:	> 20

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Rheumatoid Factor.

LIMITATIONS OF PROCEDURE

The absence of rheumatoid factor does not rule out rheumatoid arthritis. Rheumatoid Factor may appear transiently during various infections. The Rheumatoid Factor IgM ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.