Mouse Autoimmune ELISA kits available from ADI

5100 Mouse Anti-dsDNA Ig's (G+A+M) ELISA, qualitative
5110 Mouse Anti-dsDNA Ig's (G+A+M) ELISA Kit, quantitative
5120 Mouse anti-dsDNA IgG-specific ELISA Kit, quantitative
5130 Mouse anti-dsDNA IgM-specific ELISA Kit, quantitative

5200 Mouse Anti-Nuclear Antibodies (ANA) Ig's (G+A+M) ELISA Kit, qualitative
5210 Mouse Anti-Nuclear Antibodies (ANA) Ig's (G+A+M) ELISA Kit, quantitative

5300 Mouse Anti-ssDNA Ig's (G+A+M) ELISA Kit, qualitative
5310 Mouse Anti-ssDNA Ig's (G+A+Ml) ELISA Kit, quantitative
5320 Mouse Anti-ssDNA IgG-specific ELISA Kit, quantitative
5330 Mouse Anti-ssDNA IgM-specific ELISA Kit, quantitative

5400 Mouse Anti-Sm Ig's (G+A+M) ELISA Kit, qualitative
5405 Mouse Anti-Sm Ig's (G+A+M) ELISA Kit, quantitative
5410 Mouse Anti-nRNP Ig's (G+A+Ml) ELISA Kit, quantitative

5500 Mouse Anti-Cardiolipin Ig's (G+A+M) ELISA Kit, qualitative

5600 Mouse Anti-Histones Ig's (G+A+M) ELISA Kit, qualitative
5610 Mouse Anti-Histones Ig's (G+A+M) ELISA Kit

5700 Mouse Anti-SSA/Ro Ig's (G+A+M) ELISA Kit, qualitative
5710 Mouse Anti-SSA/Ro Ig's (G+A+M) ELISA Kit

5800 Mouse Anti-SSB/La Ig's (G+A+M) ELISA Kit, qualitative
5810 Mouse Anti-SSB Ig's (G+A+M) ELISA Kit

5900 Mouse Circulating Immune Complexes (CIC) Ig's (G+A+M) ELISA Kit, qualitative

6000 Mouse Anti-Jo Ig's (G+A+M) ELISA Kit, qualitative
6005 Mouse Anti-Jo-1 Ig's (G+A+M) ELISA Kit, quantitative

6100 Mouse Anti-Scl70 Ig's (G+A+M) ELISA Kit, qualitative
6110 Mouse anti-Scl70 Ig's (G+A+M) ELISA Kit, quantitative

6200 Mouse Anti- RF Ig's (G+A+M) ELISA Kit, quantitative

Human: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).
Monkey: IgM, IgG, IgA, CRP
Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein
Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF,
Chicken: IgG, IgM, IgY, Ovalbumin **Rabbit**: CRP, IgG
Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin
Pig: Albumin, IgG, IgM, **Dog:** CRP, IgG, IgM
Cat: IgG, IgM **Goat:** IgG **Sheep:** IgG **Turkey**: IgG

Instruction Manual No. M-6200

Mouse Rheumatoid Factor (RF) Ig's (total (A+G+M) ELISA Kit

Cat. No. 6200, 96 tests

For Semi-Quantitative Determination of Anti-RF Ig's (G+A+M) In Mouse Serum or Plasma or other biological fluids

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

ALPHA DIAGNOSTIC INTERNATIONAL

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Kit Contents: (Mouse Anti-RF Ig ELISA KIT, Cat. No. 6200)

Components	Cat #
Purified RF Coated Microwells (96 wells)	6201
Mouse Anti-RF negative serum, control 1 ml	6202
Mouse Anti-RF positive serum control (100 U/ml), 1 ml	6203
Anti-mouse IgG-HRP Conjugate (100X), 0.15 ml	6204
Sample Diluent(10X), 10ml	SD-10
Wash Solution (100X), 10 ml	WB-100
TMB Substrate Solution, 12 ml	80091
Stop Solution, 12 ml	80101
Complete Instruction Manual	M-6200

Intended Use

ADI's RF ELISA, a sandwich ELISA, provides a rapid, sensitive and semi-quantitative measurement of total IgGs RF in mouse serum, plasma or other biological fluids. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

INTRODUCTION

Rheumatoid Factors (RFs) were described as Immunoglobulins (IgGs) in rheumatoid arthritis as detected by agglutination of rabbit IgG coated erythrocytes. It was later demonstrated that RFs were IgM molecules reactive with the Fc-portion of the IgG from various species. In addition to the IgM RF, subsequent studies have identified RFs of other IgG subtypes (IgG, IgA, and IgE) in sera of patients with rheumatoid arthritis, and certain other chronic diseases. Most commercial assays measure predominantly IgM RFs. It has been observed that measurement of IgG RF may be a better indicator of rheumatoid arthritis than IgM RF. IgA RF and IgE RF have been implicated in articular disease process in rheumatoid arthritis and rheumatoid vasculitis, respectively. RFs, produced by cells within the joint space, are found in joint fluids as well. RF titer, particularly IgG RF correlate with the intensity of synovitis.

RFs can be found in serum samples from a variety of autoimmune diseases including rheumatoid arthritis, Systemic lupus erythematous, Sjogren's syndrome, mixed connective tissue disease, mixed essential cryoglobulinemia, scleroderma, subacute bacterial endocarditis, leprosy, pulmonary fibrosis, and pulmonary silicosis. RFs may also be found in patients with syphilis, viral infections, chronic liver diseases, sarcoidosis, leprosy, neoplasms, and a variety of other chronic conditions.

LIMITATION

Controls in the kits are provided only for quality control purpose and should not be used as reference or calculation of results. The negative cut-off value suggested in this kit are only arbitrary and can be redefined depending upon the nature of samples, reproducibility, and assay precision. However, addition of a factor (0.200 Abs.) to the negative control should serve as a good reference to determine RF positive samples. It is by no means an absolute measure of RF levels. Due to the complexity of samples, each laboratory must determine their own negative cut-off values. The units for positive control defined in this kit are arbitrary and can be redefined and/or compared to a secondary reference. Due to the lack of internationally defined negative and positive controls for mouse serum, each laboratory is encouraged to prepare its own secondary reference to help calculate sample values.

The RF values should be used as an adjunct to other methods of RF analyses. Certain drugs such as p-aminosalicylic acid, phenytoin, isoniazio, hydralazine, procainamide, etc. may induce autoantibody/RF formation. Positive results may also be obtained in apparently healthy patients due to a host of other factors. A positive RF result suggests certain diseases, but is not diagnostic and should be confirmed with other clinical findings.

SPECIFICITY

Mouse Total IgG RF is will detect RFs of IgG, IgA, and IgM origins. ADI also provide RF kits that are designed to detect the RFs of IgG or IgM type.

SPECIES REACTIVITY

This kit is designed for mouse samples. ADI has RF ELISA kits for human and other species.

INTERPRETATION OF RESULTS

It is recommended that each laboratory must determine its own normal and abnormal values. The following is intended to serve as general guidelines only:

RF Negative: Samples showing less absorbance (O.D.) than the calculated negative cut-off control can be considered as RF negative.

RF Positive: Samples with O.D. equal or higher than the calculated negative cut-off control can be considered as RF positive.

The approximate incidence of positive RF is 90 % in patients with rheumatoid arthritis and Sjogren's syndrome, 100% in mixed essential croyglobulinemia in human patient sample. RFs have been found in other diseases as well.

List of Publications using ADI ELISA kit #6200

Ho P	2010	J. Immunol., Jan 2010; 184: 379 - 390.
Hsu L-Y	2009	J. Exp. Med., Oct 2009; 206: 2527 - 2541.
Choi EM	2007	Journal of Applied Toxicology 27, 472–481
Choi EM	2007	Food and Chemical Toxicology, 46, 375-379
Zhang JQ	2005	J. Exp. Med., 200: 1467 - 1478
Drappa J	2003	J. Exp. Med., 198: 809 - 821.
Tolosa E	1998	Immunity 8: 67

Normal Values

There are no internationally accepted reference or standards are available for mouse anti-RFs. The standards used in this kit are referenced to an internal reference.

Most animals will contain a basal, normal levels of anti-Jo or non-specific background. We recommend that the reserachers establish the normal levels in control group and compare the values with the experimental groups.

In a limited testing of 10 normal balb/c (adult, mixed sex) serum samples at 1:100 diltuion produce anti-RF Ig's values A450= 0.200-0.505 range.

Autoimmune mice (Mrl lpr) showed elvated anti-RFs (A450>1.00)

EXPRESSION OF RESULTS

Determine the average absorbance for each duplicate. Subtract the average blanks values from all controls and samples. Since the baseline antibody levels in different animals will differ, the negative control provided in this kit may not serve as an ideal negative control for your samples. Therefore, it is strongly recommended that investigators define their own negatives and positives for each strain or various experimental groups.

Results can be expressed in negative and positive format.

Method 1

1. Determine the average Abs of blanks. Subtract this value from all controls and samples.
2. Add a factor of 0.200 to the specific Abs observed in the negative control provided in the kit or the one defined by the user.

Example: Specific Abs of –Ve control (0.060) + 0.200 = 0.260.

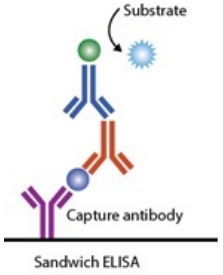
Any samples with specific Abs of >0.260 can be considered positive.

Method 2.

Most investigators find it useful to set upper limit of negative control as 2-3 times of specific Absorbance of a known negative sample. Any sample above these values may be considered as positive.

Example:
Specific Abs. Of negative sample = 0.090
Upper limit of Negative samples = 2 x 0.090 = 0.180
Therefore, all sample above >0.180 may be considered as positive.

PRINCIPLE OF THE TEST



Rheumatoid Factors (RF) ELISA kit is based on binding of RFs from serum samples to IgG antigen immobilized on microtiter wells. After a washing step, anti-mouse IgG/A/M-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and color developed. The enzymatic reaction (blue color) is directly proportional to the amount of RF present in the sample. The reaction is terminated by adding stopping solution (converts blue to yellow). Absorbance is then measured on a microtiter well ELISA reader at 450 nm. The concentration of RF in samples may be compared to a controls provided in the kit or other outside reference

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-100 µl) and multichannel pipet; Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

This ELISA Kit is intended for *in vitro research* use only. The reagents contain Proclin-300 (0.01% v/v); necessary care should be taken when disposing solutions.
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

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed to clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed, these could be stored at -20^{o}C for up to six months. Avoid repeated freezing and thawing of samples. Plasma (EDTA/Heparin) can also be used.
We recommend preparing 1:10 diluted stock of all samples (10 ul serum/plasma in 1X sample diluent). Store diluted samples at 2-4oC until test is complete or at least up to 1-week. Samples are stable in this buffer. Use this stock to prepare additional test dilutions of 1:100 or higher.

REAGENT PREPARATION FOR THE ASSAY

Sample Diluent *(10X)*. Before use, dilute 1:10 with distilled water (1ml/10ml water). Prepare 1 ml for every strip). Prepare diluent according to the requirement. Diluted conjugate can be stored for 1-2 weeks at 4oC.

Wash Buffer Concentrate *(100X)*. Before use, dilute 1:100 with distilled water. Occasionally, some salts may form crystals during storage in cold but they redissolve upon slight warming of the solution.

Anti-mouse IgG-HRP Conjugate (100X). Before use, dilute 1:100 with 1X sample diluent (10 µl/ml diluent; prepare 1 ml for every strip). Prepare only in required amount and do not store diluted conjugate beyond the assay date.

DO NOT DILUTE NEGATIVE AND POSITIVE CONTROLS PROVIDED IN THE KIT. THEY HAVE BEEN PRE-DILUTED.

STORAGE AND STABILITY

All kit components are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of manufacture under appropriate storage conditions. The unused strips should be stored tightly covered with adhesive film and with the desiccant in the bag.

Sample dilutions

It is recommended that mouse serum samples are analyzed at no less than the recommended dilution (i.e., 1:100) due to high background. Samples with A450 values >2.00 should be diluted further (for example, 1:200 or 1:500) and re-tested.

QUALITY CONTROL OF ELISA KIT

1. The negative control values must not be higher than 0.500. Higher values may indicate inappropriate washing or incorrect dilution of the conjugate or other unacceptable procedures. The test must be repeated.
2. The positive control must always have an Abs > 1.0. Lower values may indicate inappropriate washing or incorrect dilution of the conjugate or other unacceptable procedures. The test must be repeated.

If one or both controls fail to meet the above criterion, it may indicate technical problems with the assay procedures or kit components and potential causes must be investigated. Please contact ADI with and fax us the ELISA results.

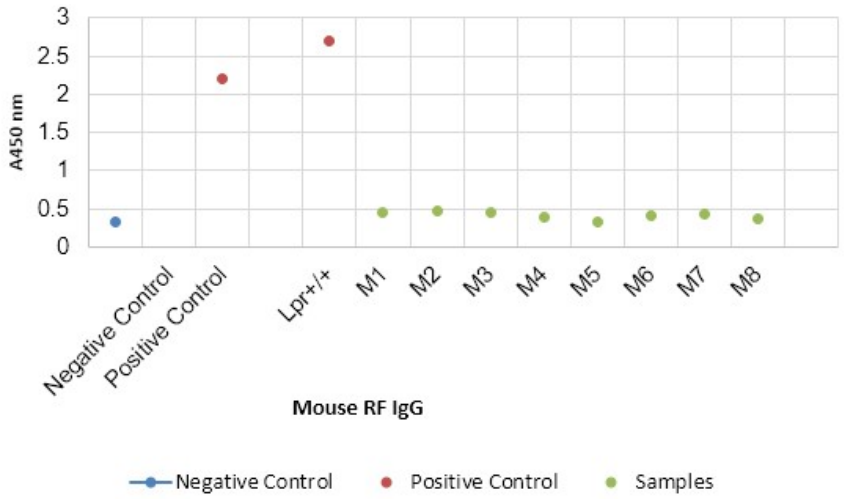
TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE). A brief summary is also given on page 7.*

1. Label, and secure the microtiter well strips to be used on the plate. **Dilute sample/conjugate** diluent 1:10 in water. **Dilute** (1:100) serum samples (5 µl serum in 500 µl of 1X sample diluent). *A total of 200 µl of diluted sample will be required to run tests in duplicate.* **Dilute (1:100) wash buffer** concentrate with distilled water. **Dilute HRP-Conjugate** (1:100) with sample diluent.
2. Pipet **100 µl** of sample diluent (Wells A1/A2 for use as blanks), negative (B1/B2), positive controls (C1/C2), and *diluted* serum samples (D1/D2, etc) into appropriate wells in *duplicate*. Mix gently, cover the plate and incubate for **30 minutes** at room temp.
3. Aspirate and **wash** the wells **4 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 values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **100 µl** of diluted enzyme conjugate into each well**.** Mix gently. Cover the plate and incubate for **30 minutes** at room temp. Aspirate and **wash** the wells **5 times** as above.
5. Add **100 µl** of TMB Substrate into each well. Mix gently. Cover the plate and incubate for **15 minutes** at room temperature.
6. **Stop** the reaction by adding **100 µl** of stop solution to all wells. Mix gently. Measure the absorbance at 450 nm using an ELISA reader (The color is stable for at least 30 min). Wells with lowest color may become clearer because of color fading with time.

Worksheet of a typical assay

Controls /samples	Wells Average Abs @450nm	Specific Abs. (Less blanks)	Results
Blanks	**A1/A2** (0.090)	-	-
-ve Control	**B1/B2** (0.250)	0.16	**Negative-**
+ve Control	**C1/C2** (1.90)	1.810	**Positive**
Sample 1 (S1)	**D1/D2** (0.280)	0.190	**Negative**
Sample 2 (S2)	**E1/E2** (0.850)	0.760	**Positive**

IMPORTANT: A complete set of blanks, negative and positive controls must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values. Negative and positive samples in the above work sheet have been determined by adding a factor of 0.200 to the negative controls. In other words, any sample above 0.260 (0.200 + 0.060) may be considered definitely positive. Users are encouraged to define their own negative and positive samples.



2-nas/6200-Mouse-RF-ELISA-Graph