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-6005

Mouse Anti-Jo Antibodies

ELISA KIT Cat. No. 6005

For Quantitative Determination of Anti-Jo Ig's (G+A+M)) In Mouse Serum

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Kit Contents: (Mouse Anti-Jo Ig ELISA KIT, Cat. No. 6005)

Components	96 tests
Purified Jo Coated strip plate (8 wells x 12 strips)	6006
Mouse anti-JO Ig's std A (0 ng/ml), 0.750ml	6007
Mouse anti-JO Ig's std B (31.25 ng/ml), 0.750ml.	6008
Mouse anti-JO Ig's std C (62.5 ng/ml), 0.750 ml.	6009
Mouse anti-JO Ig's std D (125 ng/ml), 0.750 ml.	6010
Mouse anti-JO Ig's std E (250 ng/ml), 0.750 ml.	6011
Mouse anti-JO Ig's std F (500 ng/ml), 0.750 ml.	6012
Goat Anti-mouse IgG (H+L)-HRP conjugate (100X) 0.120 ml	6013
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-6005

INTRODUCTION

Antibodies generated against the nuclear constituents are known as antinuclear antibodies (ANA). This includes autoantibodies directed against the extractable (soluble in physiological buffers) nuclear antigen or ENA. The most prominent of ANAs/ENAs are autoantibodies which binds to ds-DNA, ss-DNA, histones, ribonucleoproteins (RNP) and the SS-A, SS-B, Sm antigens, Jo-1, and Scl-70. Two antibodies, anti-dsDNA and anti-Sm, appear to occur only in SLE. Others occur in a variety of autoimmune and mixed connective tissue diseases.

A heterogeneous group of precipitating antibodies called anti-Jo-1 have been found in dermatopolymyuositis. Anti-Jo is present in approx. 25% of patients with myositis. Recently, Jo-1 has been demonstrated to be an RNA-associated 50 kDa polypeptide antigen. It has been identified as histidyl-tRNA-synthetase, the RNA being $tRNA^{his.}$.

The frequency of ANA positivity in various rheumatic diseases has been reported for SLE, rheumatoid arthritis (RA), progressive systemic sclerosis (PSS), polymyositis (PM), dermatomyositis (DM), mixed connective tissue diseases, drug-induced SLE, and Sjogren's syndrome (SS). Most of these studies are based on tedious fluorescent ANA (FANA). Other techniques such as RIA, immunodiffusion, hemagglutination, electrophoresis, and immunoblotting are also used to define antibody specificity. Recently, immunological assays (mostly ELISA) that determine the specificity of ANA have been used in studying patients with systemic rheumatic diseases.

ADI's Anti-Jo ELISA, has been developed to screen the presence of anti-Jo in **mouse serum.**

LIMITATIONS AND INTERPRETATION 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 Anti-Jo positive samples. It is by no means an absolute measure of Anti-Jo levels. Due to the complexity of samples, each laboratory must determine their own negative cut-off values and/or compared to a secondary reference. Due to the lack of universally defined negative and positive controls for mouse serum, each laboratory is encouraged to prepare its own secondary reference to help calculate sample values. The following is intended to serve as general guidelines only:

PERFORMANCE CHARACTERISTICS

Detection limit:- Based on 8 replicate determinations of the zero standards the minimum Ig's concentration detectable using this assay is 25 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

High dose hook effect:-

Ig's concentrations of up to 5 ug/ml did not show any hook effect.

Intra-assay precision:

Three mouse serum samples (mean Abs. 1.1, 0.60, and 0.40) were run in 10 replicates. The samples showed good intra-assay precision with %CV of 8-13%.

Intra-assay precision

Three mouse serum samples (mean concen. 1ug, 0.5 ug & 0.25 ug) were run in 10 replicates. The samples showed good intra-assay precision with %CV of 5.36-11.2% .

LINEARITY

Three different Mrl/lpr mouse samples were diluted (1:100, 1:200, and 1:400) and their Jo levels determined. The samples showed excellent mean recoveries of about 102% (range %).

SPECIFICITY

Jo-1 coating antigen has been immunoaffinity purified from rabbit thymus using anti-Jo-1 sepharose. Other antigens (Sm, RNP, SSA, Scl-70 and SSB) are not detected with the ELISA. Homogeneity of Jo is also verified by SDS-PAGE.

The detection antibodies used in the kit are directed against mouse IgG (H+L), it will detect total Ig's (G+A+M).

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards and samples. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate Ig's (total) concentrations. Read off the IgG concentrations of the control and treated samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:100 the values must be multiplied by 100 and the results expressed as ug/ml. If samples were diluted more than the required 1:100 dilution (i.e., 1:500) then the values multiplied by 5 or the appropriate dilution factor.

Sample dilutions

It is recommended that mouse serum samples are not analyzed at less than the recommended dilution (i.e., 1:100) due to high background. Samples with A450 values higher than the values of 1000 or more ng/ml should be diluted further (for example, 1:200 or 1:500) and re-tested.

If purified anti-Jo antibody is being tested then it will be necessary to use the actual dilution factor of the sample.

Normal Values

There are no internationally accepted reference or standards are available for mouse anti-Jo Ig's. The standards used in this kit are referenced to the anti-Jo Ig's (total) purified from a pool of mrl/pr mouse sera containing high concentration of anti-Jo Ig's.

Most animals will contain a basal, normal levels of anti-Jo. It is necessary to establish the normal levels in control group and compare the values with the experimental groups.

In a limited testing of 20 or more normal balb/c serum samples (adult , mixed sex), anti-Jo values were <70 ng/ml.

PRINCIPLE OF THE TEST

Anti-Jo ELISA kit is based on binding of Anti-Jo from serum samples to extracted nuclear antigen immobilized on microtiter wells. After a washing step, goat anti-mouse IgG-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 Anti-Jo 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 Anti-Jo in samples and control is read off the standard curve.

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 of the web site.

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

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.

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.

Goat 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).

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

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. Addition of the HRP substrate 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.

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.

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 std A (Wells A1/A2 for use as blanks), standards B-G (B1-B2-F1,F2) and then samples (G1/G2, 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 **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 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.

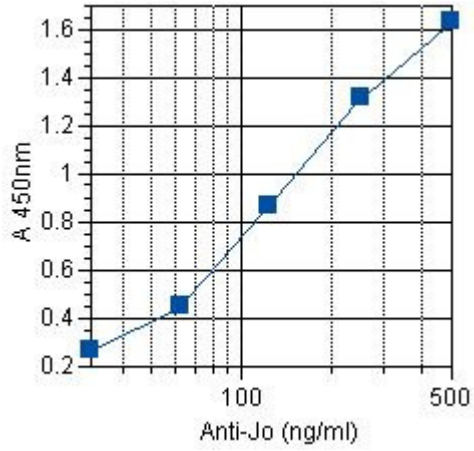
6. **Stop** the reaction by adding **100 µl** of stop solution to all wells. Mix gently. Measure the absorbance at 450 nm using an ELISA reader (The color is stable for at least 30 min). Wells with lowest color may become clearer because of color fading with time.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	*Mean A450 nm	Calculated Concn.
A1, A2	**Std A** (0 ng/ml)	0.16	
B1, B2	**Std B** (31.25 ng/ml)	0.26	
C1, C2	**Std C** (62.5 ng/ml)	0.45	
D1, D2	**Std D** (125 ng/ml)	0.86	
E1, E2	**Std E** (250 ng/ml)	1.31	
F1, F2	**Std F** (500 ng/ml)	1.63	
G1, G2	**Sample 1**	0.30	35 ng/ml

***=** Average duplicate values after deducting the std zero values.

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std.Assay (do not use for calculating values)