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 IgG (G+A+M) 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+M) ELISA Kit, quantitative

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

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

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF

Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein

Chicken: IgG, IgM, IgY, Ovalbumin, Turkey: IgG, Rabbit: CRP, IgG


Monkey: IgM, IgG, IgA, CRP

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, IPSA, 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).
Kit Contents: (Mouse Anti-histone ELISA KIT, Cat. No. 5600)

<table>
<thead>
<tr>
<th>Components</th>
<th>Cat #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified Histone Antigen Coated Microwell Strips (96 wells)</td>
<td>5 6 0 1</td>
</tr>
<tr>
<td>Mouse Anti-Histone Negative Serum Control, 1ml</td>
<td>5 6 0 2</td>
</tr>
<tr>
<td>Mouse Anti-Histone Positive Serum Control, 1ml</td>
<td>5 6 0 3</td>
</tr>
<tr>
<td>Goat Anti-mouse IgG (H+L)-HRP Conjugate (100X), 0.12 ml</td>
<td>5 6 0 4</td>
</tr>
<tr>
<td>Sample Diluent (10X), 10 ml</td>
<td>S D - 1 0</td>
</tr>
<tr>
<td>Wash Solution (100X), 10 ml</td>
<td>W B - 1 0 0</td>
</tr>
<tr>
<td>TMB Substrate, 12 ml</td>
<td>8 0 0 9 1</td>
</tr>
<tr>
<td>Stop Solution, 12 ml</td>
<td>8 0 1 0 1</td>
</tr>
<tr>
<td>Complete Instruction Manual</td>
<td>M - 5 6 0 0</td>
</tr>
</tbody>
</table>

INTRODUCTION

Antibodies generated against the nuclear constitutents 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/ENA's 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.

Histones are a group (H1, H2a, H2b, H3, and H4) of closely related, highly basic (rich in lysine and Arginine) nuclear proteins (13-22 kDa). The basic charge on histones allows a tight association with DNA. Anti-histone antibodies are even more common in SLE and probably account for the lupus erythematosus cell phenomenon. Anti-histone antibodies also arise as a side effect of treatment with various drugs and quite frequent in patients with drug induced lupus (DRL). Anti-histones have been found in class IV lupus nephritis (anti-H1), SLE (H1, H2b, H3), and rheumatoid arthritis with vasculitis. However, the presence of anti-histone antibodies are much more common in DRL (90-95%) than in SLE (20-30%).

A large number of drugs, particularly hydralazine, procainamide, isoniazide, chlorpromazine, and methyldopa, have been associated with DRL. The phenomenon of DRL has been linked with the rate of acetylation or the activity of N-acetyltransferase. Slow acetylators are more likely to induce anti-histone antibodies than fast acetylators that can rapidly metabolize drugs and reduce drug exposure.

ADI's Anti-Histone Antibodies ELISA, has been developed to screen the presence of Anti-Histone antibodies Ig's (G+A+M) 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-Histone positive samples. It is by no means an absolute measure of Anti-Histone 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:

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

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

SPECIFICITY

Purified histones used in this kit as antigen contains all bands associated with subclasses f1, f2a1, f2a2, f2b, and f3. Anti-histone antibodies, as determined by this kit, will not distinguish between these histone subisotypes.

PERFORMANCE CHARACTERISTICS

Intra-assay precision:
Three serum samples (mean Abs. 1.8, 0.73, and 0.50) were run in 10 replicates. The samples showed good intra-assay precision with %CV of 9-11%.

Inter-assay precision:
Three serum samples were run in duplicate in 8 independent assays. The samples showed good inter-assay precision (7-11 % CV).

LINEARITY

Three different Mrl/lpr mouse samples were diluted (1:100, 1:200, and 1:400) and their Anti-Histone levels determined. The samples showed excellent mean recoveries of about 102% (range 92-112%).

List of Publications using ADI's Histone ELISA Kits

Sun D., 2004 Cellular Immunol., 228, 54-65
EXPRESS 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.

QUALITY CONTROL OF ELISA KIT

1. The negative control values must not be higher than 0.300. 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 > 0.800. 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.

PRINCIPLE OF THE TEST

Anti-histone ELISA kit is based on binding of Anti-histone 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-histone 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-histone is determined by comparing it to a known reference value. Results can also be expressed as -ve or +ve when compared to a known -ve control.

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 (0.1% 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 Proclin-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 -20ºC for up to six months. Avoid repeated freezing and thawing of samples.

REAGENT PREPARATION FOR THE ASSAY

Sample/Conjugate Diluent (10X). Before use, dilute 1:10 with water (1ml/10 ml 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 solution). 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-mlgG-HRP Conjugate (100X). Before use, dilute 1:100 with 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.
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. 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°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 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 temperature.

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

<table>
<thead>
<tr>
<th>ELISA Plate format</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>E</td>
</tr>
<tr>
<td>F</td>
</tr>
<tr>
<td>G</td>
</tr>
<tr>
<td>H</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Wells Average Abs.</th>
<th>Specific Abs. (Less blanks)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanks</td>
<td>A1/A2</td>
<td>(0.090)</td>
<td>-</td>
</tr>
<tr>
<td>-ve Control</td>
<td>B1/B2</td>
<td>(0.150)</td>
<td>0.060</td>
</tr>
<tr>
<td>+ve Control</td>
<td>C1/C2</td>
<td>(1.90)</td>
<td>(1.810)</td>
</tr>
<tr>
<td>Sample 1 (S1)</td>
<td>D1/D2</td>
<td>(0.280)</td>
<td>(0.190)</td>
</tr>
<tr>
<td>Sample 2 (S2)</td>
<td>E1/E2</td>
<td>(0.550)</td>
<td>(0.460)</td>
</tr>
</tbody>
</table>

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.