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

www.4adi.com

#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 Morphogenetic 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 (PSA)
#0500 Human Alpha Fetoprotein (AFP)
#0050 Human Neuron Specific Enolase (NSE)

#0040 Human C-peptide
#0100 Human Luteinizing Hormone (LH)
#0200 Human Follicle 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 (T4)
#1650 Human free triiodothyronine (FT3)    #1700 Human T3 (total)
#1850 Human Cortisol    #1860 Human Progesterone
#1865 Human Pregnenolone    #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)

---

Human Anti-Mouse Antibodies (HAMA)

ELISA KIT Cat. # 1770

For Quantitative Determination of HAMA
In Human Serum

For In Vitro Research Use Only

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
HAMA ELISA KIT Cat. # 1770

<table>
<thead>
<tr>
<th>Kit Components, 96 tests</th>
<th>Cat #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse IgG coated strip plate (96 wells)</td>
<td>1771</td>
</tr>
<tr>
<td>HAMA Std. A (0 ng/mL), or Sample Diluent, 50 ml</td>
<td>1772A</td>
</tr>
<tr>
<td>HAMA Std B (25 ng/mL), 0.75 ml</td>
<td>1772B</td>
</tr>
<tr>
<td>HAMA Std C (100 ng/mL), 0.75 ml ml</td>
<td>1772C</td>
</tr>
<tr>
<td>HAMA Std D (400 ng/mL), 0.75 ml</td>
<td>1772D</td>
</tr>
<tr>
<td>HAMA Std E (1000 ng/mL), 0.75 ml</td>
<td>1772E</td>
</tr>
<tr>
<td>HAMA +ve control (lot sp. Values on the vial), 0.75 ml</td>
<td>1773</td>
</tr>
<tr>
<td>Anti-hlgG-HRP Conjugate, 1 1 m l</td>
<td>1774</td>
</tr>
<tr>
<td>Wash buffer concentrate (100X)</td>
<td>W-100</td>
</tr>
<tr>
<td>HRP substrate, Solution.</td>
<td>TMB1770</td>
</tr>
<tr>
<td>Stop solution (2N HCl), 1 0 m l</td>
<td>T-10</td>
</tr>
<tr>
<td>Instruction Manual</td>
<td>M-1770</td>
</tr>
</tbody>
</table>

Introduction

An exposure to foreign antigens and antibodies through laboratory exposure, blood transfusion, vaccination, or immunotherapy (infusion of polyclonal or monoclonal antibodies) may induce antibody production in humans. Circulating human antibodies reactive with animal proteins (anti-animal antibodies) are an often unrecognized and unsuspected source of interference in immunological assays, in particular two-site (sandwich) immunoassays. These antibodies include antibodies against animal immunoglobulins [e.g., human anti-mouse antibodies (HAMAs), animal albumins, and insect glycoproteins]. This type of assay interference is reported in a range of immunological assays. HAMA is probably the most common type of human anti-animal antibody. The main cause and reason for the increase in the incidence of HAMA is the use of mouse monoclonal antibodies for therapeutic and imaging purposes (intraperitoneal, intravenous, and subcutaneous routes of administration in microgram to milligram doses). A vast majority of clinical trials with mouse monoclonal antibodies, many of patients were found to have developed a HAMA response following administration of the antibody. Human anti-animal antibody responses can be of the IgG, IgA, IgM, or rarely, the IgE class. In the case of anti-animal antibodies elicited by animal immunoglobulins, the human anti-animal antibody can have anti-idiotype or anti-isotype specificity. Anti-idiotype antibodies (ab2) are directed against the hypervariable region of the immunoglobulin molecule, and anti-isotype antibodies are directed against the constant regions. Anti-anti-idiotype antibodies (ab3) can also be produced. These recognize the binding region of the anti-idiotype antibody; thus, the antigen-binding region of an anti-anti-idiotype antibody resembles the antigen that elicited the original anti-idiotype HAMA. The true number of people positive for anti-mouse antibodies is not known, and estimates vary widely (<1–80%). HAMA may neutralize the injected mAb directly by immune complex formation, which could lead to rapid clearance or to hypersensitivity reactions. HAMA remained detectable up to 2 years after the last treatment.

Exceptionally high antibody responses to HAMA (HAMA+ve) can be associated with delayed hypersensitivity reactions. These are an often unrecognized and unsuspected source of interference in immunological assays. HAMA is probably the most common type of human anti-animal antibody. The true number of people positive for anti-mouse antibodies is not known, and estimates vary widely (<1–80%). HAMA may neutralize the injected mAb directly by immune complex formation, which could lead to rapid clearance or to hypersensitivity reactions. HAMA remained detectable up to 2 years after the last treatment.

References

Hammond EH et al (1990) Transplantation 50, 776-782

PERFORMANCE CHARACTERISTICS

ACCURACY

Recovery studies were performed by mixing equal volume of test samples negative for HAMA spiked with known concentrations of HAMA. The HAMA values were measured and percentage of recovery determined.

<table>
<thead>
<tr>
<th>Initial values (ng/mL)</th>
<th>Concen spiked (ng/mL)</th>
<th>Expected values (ng/mL)</th>
<th>Observed values (ng/mL)</th>
<th>Recoveries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>22</td>
<td>11</td>
<td>11</td>
<td>100</td>
</tr>
<tr>
<td>0</td>
<td>30</td>
<td>15</td>
<td>14</td>
<td>93</td>
</tr>
<tr>
<td>0</td>
<td>52</td>
<td>26</td>
<td>23</td>
<td>89</td>
</tr>
<tr>
<td>0</td>
<td>60</td>
<td>30</td>
<td>28</td>
<td>93</td>
</tr>
<tr>
<td>0</td>
<td>90</td>
<td>45</td>
<td>42</td>
<td>93</td>
</tr>
</tbody>
</table>

2. PRECISION

Intra-assay precision

<table>
<thead>
<tr>
<th>Intra-Assay</th>
<th>Pool A</th>
<th>Pool B</th>
<th>Pool C</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Mean (ng/mL)</td>
<td>26.3</td>
<td>203.8</td>
<td>528.1</td>
</tr>
<tr>
<td>SD (ng/mL)</td>
<td>1.7</td>
<td>11.1</td>
<td>21.5</td>
</tr>
<tr>
<td>CV%</td>
<td>6.5</td>
<td>5.5</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Inter-assay precision

<table>
<thead>
<tr>
<th>Intra-Assay</th>
<th>Pool A</th>
<th>Pool B</th>
<th>Pool C</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Mean (ng/mL)</td>
<td>24.1</td>
<td>203.5</td>
<td>523.6</td>
</tr>
<tr>
<td>SD (ng/mL)</td>
<td>2.0</td>
<td>12.4</td>
<td>22.2</td>
</tr>
<tr>
<td>CV%</td>
<td>8.3</td>
<td>6.1</td>
<td>4.2</td>
</tr>
</tbody>
</table>
**WORKSHEET OF TYPICAL ASSAY**

<table>
<thead>
<tr>
<th>Wells</th>
<th>Stds/samples</th>
<th>Mean (A_{450}) nm</th>
<th>Calculated Conc (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>Std. A (0 ng/mL)</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>B1, B2</td>
<td>Std. B (25 ng/mL)</td>
<td>0.139</td>
<td></td>
</tr>
<tr>
<td>C1, C2</td>
<td>Std. C (100 ng/mL)</td>
<td>0.385</td>
<td></td>
</tr>
<tr>
<td>D1, D2</td>
<td>Std. D (400 ng/mL)</td>
<td>1.440</td>
<td></td>
</tr>
<tr>
<td>E1, E2</td>
<td>Std. E (1000 ng/mL)</td>
<td>2.59</td>
<td></td>
</tr>
<tr>
<td>F1, F2</td>
<td>+ve control</td>
<td>0.601</td>
<td>162 x 100</td>
</tr>
</tbody>
</table>

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.

**PRINCIPLE OF THE TEST**

HAMA ELISA kit is based on binding of HAMA from samples to antigen immobilized on the microtiter well plates. Bound antibodies are detected by anti-human IgG-enzyme (horseradish peroxidase) conjugate. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of HAMA 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 HAMA in samples and control is read off the standard curve.

**MATERIALS AND EQUIPMENT REQUIRED**

Adjustable micropipet (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

**PRECAUTIONS AND SAFETY INSTRUCTIONS**

The Alpha Diagnostic International HAMA ELISA kit is intended for in vitro research use only. The reagents contain thimerosal (0.05%) as preservative; necessary care should be taken when disposing solutions. The stds./controls sera contain human serum that has been shown to be negative for HbsAg, HCV, and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled at biosafety level 2, as recommended for any potentially infectitious human serum or blood specimen in the CDC/NIH Manual, "Biosafety in microbiological and biomedical laboratories, 1984".

All waster material should be properly disinfected before disposal. Avoid contact with the stop solution (2N HCl).

**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 can not be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

**REAGENTS PREPARATION FOR THE ASSAY**

*Samples. Before use, dilute 1:100 (10 ul in 990 ul sample diluent). It is possible to take less for dilution, but it may increase error.*

*Wash Buffer- Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter). store at 4oC for long term.*

*Do not dilute standards.*

All other reagents are ready to use.
STORAGE AND STABILITY
The microtiter well plate and all other reagents, if unopened, 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 shipping under appropriate storage conditions. After opening the kit components, the shelf life is approx. 2 months.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).
Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 ul of wash buffer to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

1. Dilute serum samples 1:100 using diluted sample diluent (10 ul sample in 990 ul of diluent). **Do not dilute standards.**
2. Label or mark the microtiter well strips to be used on the plate.
3. Pipet 25 ul stds, controls and diluted samples into appropriate wells.
4. **Note:** for ease of loading samples it is recommended that a second uncoated microwell plate should be used as a reservoir. This enables standards or samples to be transferred quickly to the ELISA plate using multichannel pipet.
5. Pipet 100 ul of Ab-enzyme conjugate into each well. **Mix gently.**
6. Cover the plate and incubate for 30 minutes at room temperature.
7. Aspirate and wash the wells 5 time with 1X 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.
8. Dispense 100 ul TMB substrate per well. **Mix gently.**
9. Cover the plate and incubate for 15 minutes at room temperature. Blue color develops.
10. Stop the reaction by adding 50 ul of stopping solution to all wells at the same timed intervals as in step 8. Mix gently. Blue color turns yellow.
11. Measure the absorbance at 450 nm using an ELISA reader. 570 nm filter can be used as reference filter. A well with the substrate only can be used as blank. Color is stable for at least 1 hr after stopping.

**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 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°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 wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES
Samples containing more than 200 ng/mL HAMA should be first diluted with the assay buffer and then diluted normally 1:200 (along with the standards and control), as described in the assay procedure. The results obtained should be multiplied by the appropriate first dilution factor. It is possible to use, normal saline or PBS for sample dilution if larger volumes of samples are taken for analysis without diluent is required.

CALCULATION OF RESULTS
Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on linear scale or 3-cycle log-log graph paper by plotting net absorbance values of standards against appropriate HAMA concentrations. Read off the HAMA concentrations of the control and patient samples. Multiply the values by 100 or the dilution factor of the samples if samples were diluted by a factor other than 1:100.

SPECIFICITY
In studies of the ADI HAMA Kit the presence of rheumatoid factor as well as other autoantibodies (anti-DNA and anti-ENA) were shown not to interfere with the accurate detection and quantitation of HAMA. The presence of anti-viral (CMV, HSV and Hepatitis A and B) and anti-Toxoplasma antibodies in either the acute or convalescent phase of infection were also shown not to interfere with the HAMA kit (see data in table on reference range). **NOTE:** Circulating mouse immunoglobulin (Ig) in samples may interfere with the accurate detection and HAMA quantitation. In these samples the mouse Ig should be quantitated (ADI Cat # 6320)

REFERENCE RANGE
Various samples were tested for the presence of HAMA and the results are given below.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean Abs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1. Healthy donors</td>
<td>50</td>
<td>0.081</td>
</tr>
<tr>
<td>Group 2. Rheumatic disease patients</td>
<td>30</td>
<td>0.080</td>
</tr>
<tr>
<td>Group 3. Acute infection patients</td>
<td>30</td>
<td>0.088</td>
</tr>
<tr>
<td>Group 4. Patients treated with mouse</td>
<td>12</td>
<td>0.559</td>
</tr>
<tr>
<td>monoclonal antibodies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
