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IVD



See external label



2°C-8°C



Σ=96 tests

REF

Cat # 1027Z

Enzyme Immunoassay Human Anti-Mouse Antibody

HAMA

Cat # 1027Z

For *in vitro* Research Use Only

INTRODUCTION

Human Antibodies to mouse immunoglobulins are known as HAMA (Human Anti-Mouse Antibody)¹⁻¹⁰. The Diagnostic Automation Inc. HAMA kit is an enzyme immunoassay that provides for the sensitive detection and quantitation of HAMA. The HAMA kit is completed with all reagents ready to use including standards and a positive control. Test samples are diluted and quantitated in ug HAMA/mL of sample from the standard curve.

Test Samples and an enzyme-linked mouse IgG are added to coated wells of a 96-well microplate. A chromogen-substrate is added and the enzyme reaction stopped with the absorbance read at 450 nm. The assay can be completed in less than one hour.

MATERIAL PROVIDED

1. Micro-well Strips: 8x12 microplate strips coated with Mouse- IgG, 96 wells.
2. Enzyme Conjugate (11 mL): Mouse IgG conjugated to horseradish peroxidase
3. Sample Diluent or Zero Standard (50 mL).
4. Reference Standard Set (0.5 mL/each): Calibrated to 25, 100, 400, and 1000 ng/mL in BSA-containing diluent.
5. HAMA positive Control (0.5 mL)
6. Concentrated washing Buffer (20x) (50 mL)
7. Solution A (11 mL): Buffer solution containing hydrogen peroxide.
8. Solution B (11 mL): Buffer solution containing tetramethylbenzidine.

9. Stop Solution: 2N HCl.
10. Well holder: for securing wells.
11. Package Insert.

*** The reagents except Solution A and Solution B contains 0.01% Thimerosal as a preservative***

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micro-well reader
2. Disposal tips and pipettor for measuring 25L uL, 50 uL and 100 uL.

WARNING AND PRECAUTION

1. DAI HAMA QUANTITATIVE is designed for in Vitro Research Use only.
2. The Components in the kits are intended for usage as an integral unit. The components from different lots should not be mixed, and not be used beyond expiration date.
3. The material should be used in a designated work area, the bench surface should be cleaned with detergent and the contaminated materials should be disposed properly.
4. Some components have been tested using FDA-approved methods and has been found negative for antibody to human immuno-deficiency virus (HIV-I, HIV-II), antibody to Hepatitis C and Hepatitis B surface antigen (HBsAg). No known test method can offer total assurance that HIV-I, HIV-II, Hepatitis B & C virus or other infectious agents are absent. Handle these reagents as if they were potentially infectious. Information on handling human serum is provided in the CDC/NIH manual A Biosafety in Microbiological and Biomedical Laboratories@ (U.S.A. HHS publication No. (NIH 88-8395.)
5. Avoid microbial contamination of reagents when removing aliquots from the vials.

STORAGE AND STABILITY

1. Store the kits at 2-8°C in a refrigerator.
2. Keep micro-wells in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit. Solution A and Solution B should be colorless; if the solution turns blue, it must be replaced. Do not expose these reagents to strong light during storage or usage.

SPECIMEN AND COLLECTION

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation at room temperature. Serum is required for MAGIWEL™ HAMA assay, and do not add sodium azide as preservative. Samples should not be stored at room temperature or 4°C for more than 24 hours. Serum samples are recommended to be frozen for longer storage. Avoid repeated freezing and thawing of serum samples. Mild hemolysis and lipemia have been shown not to interfere with the results. Specimens that are grossly lipemic, hemolyzed or contaminated may interfere and should not be used.

PREPARATION FOR ASSAY

1. Bring all reagents and samples to room temperature (20° C - 25° C) and shake gently before beginning the test. Have all reagents and samples ready before the start of the assay. Once the test is begun it must be performed without any interruption to get the most reliable and consistent results.
2. Use new disposable tips for each specimen.

ASSAY PROCEDURE

Note: Standards and Positive Control are ready to use, do not dilute.

Sample dilution: Add 10 uL of test sample to sample diluent in the tube and mix (1:101 dilution).

It is recommended that samples, standards and positive control be run in duplicate.

1. Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.
2. Dispense 25 uL of references, controls or diluted serum samples (1:101) into the appropriate wells.
3. Dispense 100 uL of enzyme conjugate into wells.
4. Incubate for 30 minutes at room temperature.
5. Remove incubation mixture and rinse the wells 5 times with diluted washing buffer.
6. Dispense 100 uL of solution A and 100 uL of solution B into each well.
7. Incubate for 15 minutes at room temperature.
8. Stop reaction by adding 50 uL of 2 N HCl to each well.
9. Blank with the substrate only well(s) and read absorbance at 450 nm for dual wavelength readers use 570 nm as the reference wavelength.

PROCEDURE NOTE

1. Wash the microwells and remove water thoroughly.
2. Pipet all reagents and samples into bottom of the well. Vortex-mixing or shaking is not required.
3. Absorbance is a function of the time and temperature of incubations. It is recommended to have reagents, samples and needed wells ready for ensure the equal elapsed time for each pipetting without interruption.
4. For the same reason run no more than 20 patient samples with a set of reference standards in duplicate for each assay.

QUALITY CONTROL

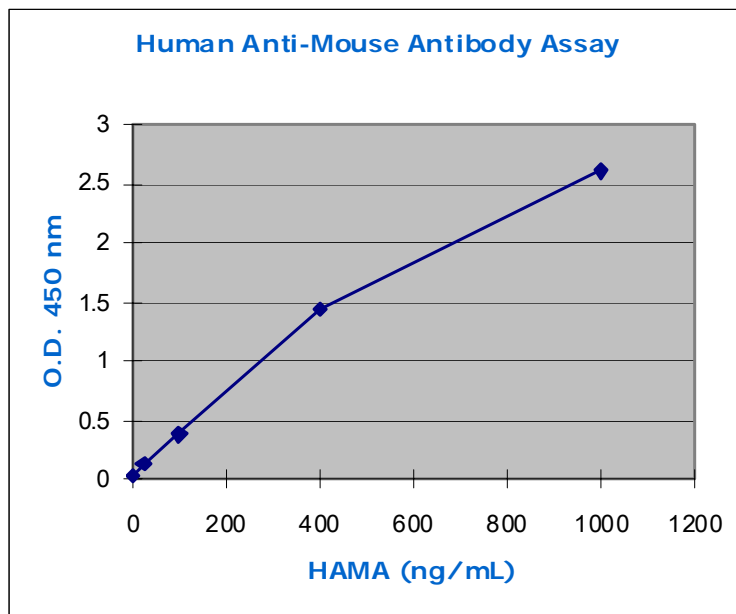
Good laboratory practices include the use of control specimens to ensure that all reagents and protocols are performing properly. DAI HAMA Assay kit does include serum control.

CALCULATION OF RESULTS

1. Plot the concentration (X) of each reference standards against its absorbance (Y) on a 3 cycle log-log paper (see below) paper.
2. Obtain the HAMA value of patient by reference to the standard curve as follows: (These data are for

demonstration purpose only and must not be used in place of data generated for each assay).

Well No.	Descrip. (ng/mL)	Absorbance (450 nm)	HAMA (ng/mL)
A1	0	0.028	
B1	(Blank)	0.027	
A2	25	0.128	
B2		0.134	
A3	100	0.377	
B3		0.382	
A4	400	1.441	
B4		1.438	
A5	1000	2.590	
B5		2.624	
A6	CONTROL	0.053	8.7 x 10 ¹
B6		0.052	8.5 x 10 ¹
A7	CONTROL	0.608	162.6 x 10 ¹
B7		0.611	163.5 x 10 ¹



Determine the level of HAMA (ng/mL) in the test sample by reading of the standard curve and multiply result by dilution factor (x101). Samples with absorbances greater than the highest standard should be diluted further and retest to quantitate the HAMA. Record results in ug HAMA/mL of sample.

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.

Initial Values ng/mL	Conc. Spiked ng/mL	Expctd Values ng/mL	Obsrvd Values ng/mL	Recvry (%)
0	22	11	11	100
0	30	15	14	93
0	52	26	23	89
0	60	30	28	93
0	90	45	42	93

PRECISION

Intra-assay and inter-assay coefficient of variation were evaluated at three different pooled serum samples.

Intra-assay	Pool A	Pool B	Pool C
N	18	18	18
Mean (ng/mL)	26.3	203.6	528.1
S.D. (ng/mL)	1.7	11.1	21.5
C.V. (%)	6.5	5.5	4.1

Inter-assay	Pool A	Pool B	Pool C
N	18	18	18
Mean (ng/mL)	24.1	203.5	523.6
S.D. (ng/mL)	2.0	12.4	22.2
C.V. (%)	8.3	6.1	4.2

SPECIFICITY

In studies of the DAI 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 DAI 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.

REFERENCE RANGE

Various samples were tested for presence of HAMA and the results are listed below.

Groups	N	Mean Abs	Abs. Range
Group 1 Healthy Controls	50	0.081	0.043-0.119
Group 2 Rheumatic Disease patients	30	0.080	0.069-0.092
Group 3 Patients with Acute Infections	30	0.088	0.069-0.110
Group 4 Patients treated with Mouse Monoclonal Antibodies	12	0.559	0.010-2.500

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