# DRG® HAMA (EIA-4195)

**Revised 8 Jan. 2009 (Vers. 3.0)**

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***For Research Use Only***

## INTRODUCTION

Human Antibodies to mouse immunoglobulins are known as HAMA (Human Anti-Mouse Antibody) [1-10].
The HAMA ELISA kit is an enzyme immunoassay that provides materials 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 µg 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 TMB-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 (0.75 mL/each):
   Calibrated to 25, 100, 400, and 1000 ng/mL in BSA-containing diluent.
5. HAMA **positive Control** (0.75 mL)
6. Concentrated **Wash Buffer** (**100x**) (10 mL)
7. **TMB Solution** (11 mL): Buffer solution containing hydrogen peroxide and TMB
8. **Stop Solution** (10 mL): 2N HCl.
9. **Well holder**: for securing wells.
10. Package Insert.

*** The reagents except TMB Solution contain 0.01% Thimerosal as a preservative***

## MATERIALS REQUIRED BUT NOT PROVIDED

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

## WARNING AND PRECAUTION

1. The HAMA ELISA 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. TMB Solution 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 the HAMA ELISA, 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 µL 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 µL of references, controls or diluted serum samples (1:101) into the appropriate wells.
3. Dispense 100 µL 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 µL of TMB solution into each well.
7. Incubate for 15 minutes at room temperature.
8. Stop reaction by adding 50 µL 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. The HAMA ELISA 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).

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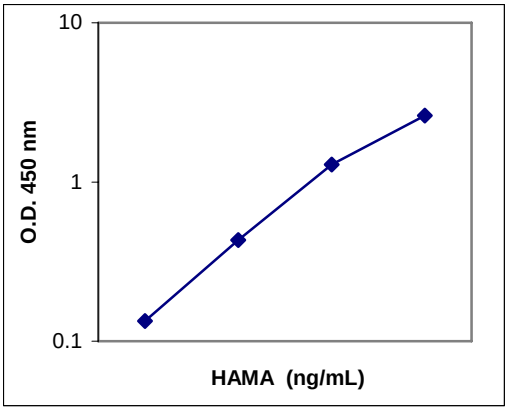
| Well No. | Description (ng/mL) | Absorbance (450 nm) | HAMA (ng/mL) |
|---|---|---|---|
| A1<br>B1 | 0<br>(Blank) | 0.028<br>0.027 | |
| A2<br>B2 | 25 | 0.128<br>0.134 | |
| A3<br>B3 | 100 | 0.377<br>0.382 | |
| A4<br>B4 | 400 | 1.441<br>1.438 | |
| A5<br>B5 | 1000 | 2.590<br>2.624 | |
| A6<br>B6 | CONTROL | 0.053<br>0.052 | 8.7 x 101<br>8.5 x 101 |
| A7<br>B7 | CONTROL | 0.608<br>0.611 | 162.6 x 101<br>163.5 x 101 |




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 µg 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) | Concentration Spiked (ng/mL) | Expected Values (ng/mL) | Observed Values (ng/mL) | Recovery (%) |
|---|---|---|---|---|
| 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 this HAMA ELISA 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 ELISA 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**<br>Healthy Controls | 50 | 0.081 | 0.043-0.119 |
| **Group 2**<br>Rheumatic Disease patients | 30 | 0.080 | 0.069-0.092 |
| **Group 3**<br>Patients with Acute Infections | 30 | 0.088 | 0.069-0.110 |
| **Group 4**<br>Patients treated with Mouse Monoclonal Antibodies | 12 | 0.559 | 0.010-2.500 |

## REFERENCES

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## Symbols used with DRG Assays

| Symbol | English | Deutsch | Français | Español | Italiano |
|---|---|---|---|---|---|
|  | Consult instructions for use | Gebrauchsanweisung beachten | Consulter les instructions d'utilisation | Consulte las instrucciones de uso | Consultare le istruzioni per l'uso |
| CE | European Conformity | CE-Konfirmitäts-kennzeichnung | Conformité aux normes européennes | Conformidad europea | Conformità europea |
| IVD | In vitro diagnostic device | In-vitro-Diagnostikum | Usage Diagnostic in vitro | Para uso Diagnóstico in vitro | Per uso Diagnostica in vitro |
| RUO | For research use only | Nur für Forschungszwecke | Seulement dans le cadre de recherches | Sólo para uso en investigación | Solo a scopo di ricerca |
| REF | Catalogue number | Katalog-Nr. | Numéro de catalogue | Número de catálogo | Numero di Catalogo |
| LOT | Lot. No. / Batch code | Chargen-Nr. | Numéro de lot | Número de lote | Numero di lotto |
|  | Contains sufficient for <n> tests/ | Ausreichend für "n" Ansätze | Contenu suffisant pour "n" tests | Contenido suficiente para <n> ensayos | Contenuto sufficiente per "n" saggi |
|  | Storage Temperature | Lagerungstemperatur | Température de conservation | Temperatura de conservación | Temperatura di conservazione |
|  | Expiration Date | Mindesthaltbarkeits-datum | Date limite d'utilisation | Fecha de caducidad | Data di scadenza |
|  | Legal Manufacturer | Hersteller | Fabricant | Fabricante | Fabbricante |
| Distributed by | Distributor | Vertreiber | Distributeur | Distribuidor | Distributore |
| Content | Content | Inhalt | Conditionnement | Contenido | Contenuto |
| Volume/No. | Volume / No. | Volumen/Anzahl | Volume/Quantité | Volumen/Número | Volume/Quantità |

| Symbol | Portugues | Dansk | Svenska | Ελληνικά |
|---|---|---|---|---|
|  | Consulte as instruções de utilização | Se brugsanvisning | Se bruksanvisningen | Εγχειρίδιο χρήστη |
| CE | Conformidade com as normas europeias | Europaeisk overensstemmelse | Europeisk överensstämmelse | Ευρωπαϊκή Συμμόρφωση |
| IVD | Diagnóstico in vitro | In vitro diagnostik | Diagnostik in vitro | in vitro διαγνωστικό |
| RUO | | | | |
| REF | Catálogo n.º | Katalognummer | Katalog nummer | Αριθμός καταλόγου |
| LOT | No do lote | Lot nummer | Batch-nummer | Αριθμός Παρτίδος |
|  | | Indeholder tilsttrækkeligt til "n" test | Innehåller tillräckligt till "n" tester | Περιεχόμενο επαρκές για «n» εξετάσεις |
|  | Temperatura de conservação | Opbevarings-temperatur | Förvaringstempratur | Θερμοκρασία αποθήκευσης |
|  | Prazo de validade | Udløbsdato | Bäst före datum | Ημερομηνία λήξης |
|  | Fabricante | Producent | Tillverkare | Κατασκευαστής |
| Distributed by | | | | |
| Content | Conteúdo | Indhold | Innehåll | Περιεχόμενο |
| Volume/No. | Volume/Número | Volumen/antal | Volym/antal | Όγκος/αριθ.. |