HAMA ELISA Kit

Cat. No.: DEIA1915
Pkg. Size: 96T

### Intended Use

The HAMA ELISA kit is an enzyme immunoassay that provides materials for the sensitive detection and quantitation of HAMA.

### General Description

Human Anti-Mouse Antibody (HAMA) are antibodies found in human serum which have the ability to bind to mouse immunoglobulin G (IgG). The presence of HAMA is the result of an immune reaction following an exposure to mice or other similar agent, which was able to induce the immune system to generate an antibody able to bind to mouse IgG. HAMA is commonly found in patients following treated with mouse monoclonal antibodies associated with some therapeutic or diagnostic procedures. Some human auto-antibodies, the most common of which is rheumatoid factor (RF), by virtue of their cross reactivity are able to bind mouse IgG.

### Principle of The Test

The HAMA kit is complete 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.

### Reagents And Materials 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: 2N HCl.

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

### Materials Required But Not Supplied

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

### Storage

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 Collection And Handling**

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.

**Assay Steps**

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. Use new disposable tips for each specimen.

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 (1:10dilution).

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.

**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**

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 each sample 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).

**Typical Standard Curve**

This standard curve is provided for demonstration only. A standard curve should be generated for each assay plate. 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.
Precautions

1. 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.

2. 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.

3. 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.)

4. Avoid microbial contamination of reagents when removing aliquots from the vials.

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