Instructions for Use

HAMA ELISA

Enzyme Immunoassay for the quantitative Determination of
human anti-Mouse Antibodies in Serum

For research use only. Not for use in diagnostic procedures.

Cat.-No.	:	IDC-14
Size	:	12 x 8
Storage	:	2 - 8°C

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Contents

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1. Introduction

The HAMA Enzyme Immunoassay Kit provides materials for the determination of human anti-mouse antibodies (HAMA) in serum. This assay is intended for research use only, not for use in diagnostic procedures.

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 injection of murine monoclonal antibodies associated with some medical 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. The HAMA assay has been designed to not be affected by rheumatoid factor. The HAMA assay measures HAMA across a wide dynamic range and it is not necessary to predilute human serum or plasma samples before the HAMA assay. However, if an elevated HAMA sample is diluted with the HAMA zero calibrator the HAMA assay has been demonstrated to yield a linear dilution of HAMA.

2. Principle of the Test

This assay is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with an antibody, directed towards an epitope of an antigen molecule.
An aliquot of unknown serum is incubated in the coated well with enzyme conjugated second antibody (E-Ab), directed towards a different region of the antigen molecule. After incubation the unbound E-Ab is washed off. The amount of bound E-Ab is proportional to the concentration of antigen in the sample. After adding the substrate solution, the intensity of colour developed is proportional to the antigen concentration in the sample. The measured ODs of the standards are used to construct a calibration curve against which the unknown samples are calculated.

3. Precautions

- The assay calibrators and controls are of human origin and have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All standards, however, should be treated as potential biohazards in use and for disposal.
- The assay reagents contain sodium azide or thimerosal which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
- The stop solution contains H_2SO_4. If it comes into contact with skin, wash thoroughly with water and seek medical attention. Since the H_2SO_4 used to terminate the colour reaction is corrosive, the instrumentation employed to dispense it should be thoroughly cleaned after use.
- This kit is for research use only.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidical soap and copious amounts of water.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents, and wash hands thoroughly afterwards. Microbial contamination of reagents or specimens may give false results.

4. Storage and Stability

Store all reagents at 2 - 8°C and use before expiry date.

Unused microtiter strips must always be stored at 2 - 8°C in the resealable bag provided. Allow reagents and required number of strips to reach room temperature prior to use.

5. Contents of the Test

5.1. Microtiter strips 12 Strips

8 wells each, ready to use
coated with mouse IgG.

5.2. Standards (1 - 5) 5 vials

1 ml each (zero standard: 10 ml), ready to use
anti-mouse IgG containing 0.01% merthiolate as
a preservative. The exact concentration is
indicated on the vials.

Concentrations:

Standard	1	2	3	4	5
Conc. (ng/ml)	0	37.5	75	150	300

5.3. Controls 2 vials

1 ml each, ready to use
anti-mouse IgG containing 0.01% merthiolate
as a preservative.

5.4. Enzyme Conjugate 1 vial

5 ml, ready to use
IgG conjugated to the enzyme horseradish peroxidase
containing 0.01% merthiolate as a preservative.

5.5. TMB Substrate Solution 1 vial

10 ml, ready to use
TMB substrate containing 0.01% merthiolate
as a preservative.

5.6. TMB Stop Solution 1 vial

10 ml, ready to use
1 N sulfuric acid. Caution: corrosive!

5.7. Wash Buffer, concentrated (10x) 1 bottle
30 ml, concentrated
containing a detergent and 0,1% merthiolate as a preservative.

Material required but not provided

- Automatic pipettes to dispense 25, 50, 100 and 400 µl (a multichannel pipetting device such as Titertek is suitable for adding reagents to the wells.).
- Volumetric measuring cylinder 10 - 50 ml.
- Test tubes for sample dilution.
- Distilled or deionized water.
- Microtiterplate spectrophotometer (ELISA reader) with 450 nm filter.

6. Preparation of Specimen and Storage

This assay may be performed with serum samples. Avoid grossly hemolysed or lipemic samples.
The samples should be stored at 2 - 8°C soon after collection. Samples may be stored frozen (-20°C or lower) for longer period of time. Do not thaw frozen samples in a hot water bath, thaw at room temperature and mix by gentle swirling or inversion.

7. Preparation of Samples and Reagents

7.1. Samples
Prepare a 1 to 2 dilution of all samples with zero standard.

7.2. Wash Buffer
Prepare a 1 to 10 dilution of desired volume of the 10x concentrated wash buffer with distilled or deionized water.

8. Assay Procedure

GENERAL REMARKS:

It is recommended to use control samples according to state and federal regulations. The use of control sera or plasma is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.

Once the test has been started, all steps should be completed without interruption.

Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination. For the dispensing of the TMB substrate solution and the TMB stop solution avoid pipettes with metal parts.

Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

8.1. Pipette **50 µl enzyme conjugate** into each well.

8.2. Add **50 µl standards, control** or **diluted sample**.

8.3. Mix well by a slow shaking of the wells for a few seconds and incubate for **60 minutes at room temperature**.

8.4. Aspirate and wash the wells 5x with **diluted wash buffer** (**0.4 ml** each wash). Wash the wells 5x with **distilled water** (**0.4 ml** each wash). Tap the wells gently and blot the rims to remove all residual droplets.

8.5. Pipette **100 µl TMB substrate solution** into each well.

8.6. Mix well by a slow shaking of the wells for a few seconds and incubate for **30 minutes at room temperature**.

8.7. Add **100 µl TMB stop solution** into each well and mix well by a slow shaking of the wells for a few seconds.

8.8. Determine the absorbance of all the wells in a spectrophotometer at **450 nm** (Reference wavelength: 630 nm).

9. Calculation of Results

Any ELISA reader capable of determining the absorbance at 450 ± 10 nm may be used. The antigen concentration of each sample is obtained as follows:

Using linear-linear or semi log graph paper construct an standard curve by plotting the average absorbance (Y) of each reference standard against its corresponding concentration (X) in ng/ml.

Use the average absorbance of each sample to determine the corresponding antigen value by simple interpolation from this standard curve, multiplying by the initial sample dilution, if necessary. The values are multiplied by 2 (dilution factor) to achieve the final HAMA result.

Alternatively the use of a data reduction program is possible, and results can be calculated with normal programs such as 4 parameter, spline-fit, logit-log.

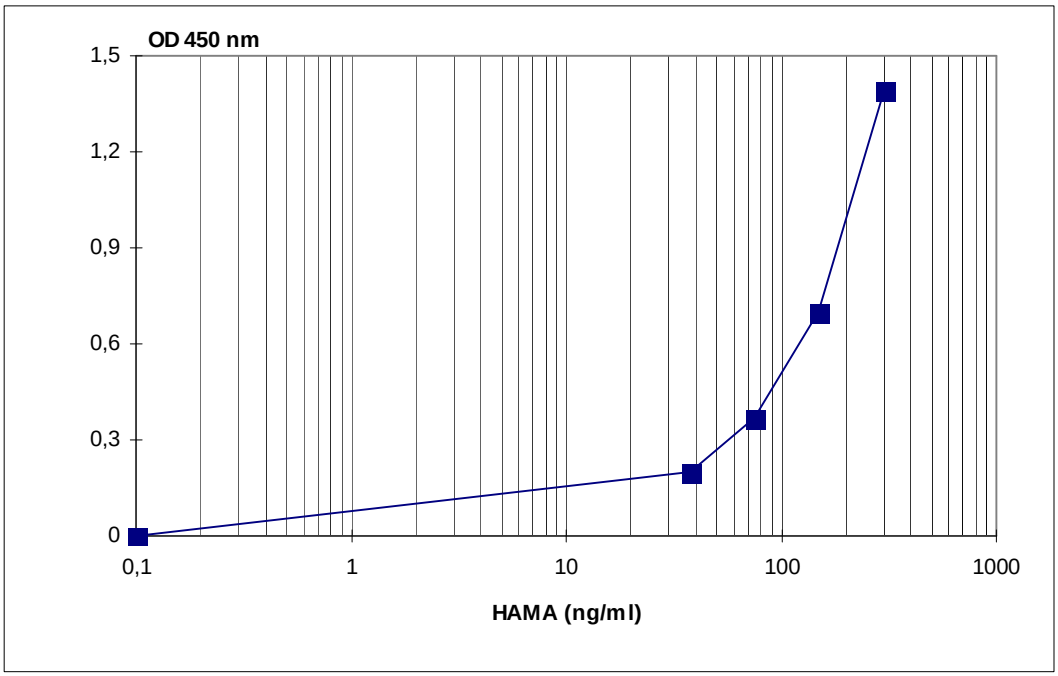
Any sample reading greater than the highest standard should be diluted appropriately with zero standard and reassayed.

Do not use this calibration curve. In the laboratory the standard curve should be established in each assay run.

Standard	HAMA Conc. (ng/ml)	Mean Absorbance (OD 450 nm)	Corrected Absorbance (OD 450 nm)
1	0	0.060	-
2	37.5	0.263	0.203
3	75	0.426	0.366
4	150	0.761	0.701
5	300	1.453	1.393

Typical Standard Curve

HAMA - ELISA



10. Expected Values

Reference range study was conducted in 120 human serum samples using the HAMA ELISA kit. The following results were obtained:

Mean: 51.1 ng/ml Standard dev. ± 38.09 ng/ml
Range: 0 - 188 ng/ml

In approximately 9% of a normal population, pre-existing HAMA reactivity have been detected.

Each laboratory should establish its own reference values to confirm with the characteristics of the population that is being tested.

11. Limitations of use

This assay is for research use only.

In some individuals the presence of potentially cross-reactive heterophilic antibodies may give positive HAMA response.
Samples with values greater than 300 ng/ml should be diluted with zero standard and reassayed.
Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

Azide and thimerosal at concentrations higher than 0.1 % interfere in this assay. Therefore control sera or samples containing higher concentrations of these components may give false results.

Reagents from different kits or lots should not be mixed, due to possible different shipping or storage conditions.

Any improper handling of samples or modification of this test might influence the results.

Interference caused by improper sample handling are explained in chapter ‘Specimen Collection and Storage’.

12. Warranty

Any modification of this test as well as exchange or mixture of any components from different lots might influence the results. In such cases there is no claim for a replacement.

13. References

1. Boerman OC, Segers MFG, Poels LG, Kenemans P, Thomas CMG. Heterophilic antibodies in human sera causing falsely increased results in the CA 125 immunofluorometric assay. Clin. Chem., **36**: 888-891 (1990).

2. Dillman RO. Human anti-mouse and anti-globulin responses to monoclonal antibodies. Antibody, immunoconjugates and radiopharmaceuticals, **1**: 1-15 (1990).
3. Kricka LJ, Schmerfeld-Pruss D, Senior M, Goodman DBP, Kaladas P. Interference by human anti-mouse antibody in two site immuno-assays. Clin. Chem., **36**: 892-894 (1990).
4. Hansen HJ, LaFontaine G, Newman ES, Schwartz MK, Malkin A, Mojzisik K, Martin EW, Goldberg DM. Solving the problem of antibody interference in commercial ”sandwich” -type immunoassays of carcinoembryonic antigen. Clin. Chem., **35**: 146-151 (1989).
5. Van Kroonenburgh MJPG, Pauwels EKJ. Human immunological response to mouse monoclonal antibodies in the treatment or diagnosis of malignant diseases. Nucl. Med. Comm., **9**: 919-930 (1988).
6. Wright jr. GL. Application of monoclonal antibodies to the study of human cancer. In: Wright jr. G.L., ed.: Monoclonal antibodies and cancer. New York-Basel: Marcel Dekker Inc., **23**: 1-29 (1988).
7. Brady LW, Woo DV, Heindel ND, Markoe MD, Koprowsky MD. Therapeutic and diagnostic uses of modified monoclonal antibodies. Int. J. Radiat. Oncol. Biol. Phys., **13**: 1535-1544 (1987).
8. Courtenay-Luck NS, Epenetos AA, Winearls CG, et al. Preexisting human anti-murine immunoglobulin reactivity due to polyclonal rheumatoid factors. Cancer Res., **47**: 4520-4525 (1987).
9. Dillman RO, Beauregards JC, Halpern SE, Clutter M. Toxicities and side effects associated with intravenous infusions of murine monoclonal antibodies. J. Biol. Res. Mod., **5**: 73-84 (1986).
10. Jaffers JJ, Fuller TC, Cosimi AB, Russell PS, Winn HJ, Colvin RB. Monoclonal antibody therapy. Transplantation, **41**: 572-578 (1986).
11. Shawler DL, Bartholomew RM, Smith LM, Dillman RO. Human response to multiple injections of murine monoclonal IgG. J. Immunol., **135**: 1530-1535 (1985).

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