Human anti-mouse antibody (HAMA) ELISA Kit

Enzyme Immunoassay for the quantification of Human anti-mouse antibody (HAMA) in serum.

Catalog number: ARG80932

Package: 96 wells

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

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INTRODUCTION

Human anti-mouse antibody or human anti-murine antibody (HAMA) is an antibody found in humans which reacts to immunoglobins found in mice.

Antibody treatment is a type of therapy that is used to treat certain types of cancer and immune disorders.

For several decades, and until recently, mice were used extensively in the production of monoclonal antibodies (MAbs). But the treatments were not as effective as doctors had hoped. One problem was that patients reacted to the mouse antibodies as if they were a foreign substance, and created a new set of antibodies to the mouse antibodies. Doctors have termed this the "HAMA response," referring to the development of Human Anti-Mouse Antibodies (HAMA). The HAMA response is essentially an allergic reaction to the mouse antibodies that can range from a mild form, like a rash, to a more extreme and life-threatening response, such as kidney failure. HAMA can also decrease the effectiveness of the treatment, or create a future reaction if the patient is given a subsequent treatment containing mouse antibodies. [Provide by Wikipedia: Human anti-mouse antibody]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A purified mouse IgG has been pre-coated onto a microtiter plate. Standards, and samples are pipetted into the wells and any HAMA (Human Anti-Mouse

Antibody) present is bound by the immobilized mouse IgG. The simultaneously added HRP-Conjugate to each well and incubate. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of HAMA bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of HAMA in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Pre-coated microplate (coat with mouse IgG)	8 X 12 strips	4°C
Standards (8000ng/vial, rabbit anti mouse IgG)	2 vial (lyophilized)	4°C
HRP Conjugate (mouse IgG conjugate HRP)	12 mL (ready to use)	4°C
Diluent Buffer	25 mL (ready to use)	4°C
20X Wash Buffer	60 mL	4°C
TMB substrate A	10 mL	4°C
TMB substrate B	10 mL	4°C (protect from light)
STOP solution	15 mL (ready to use)	4°C

Store all other components at 2-8°C. Use the kit before expiration date. Opened kits retain activity for 8 weeks if stored as described above.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Sodium hypochlorite solution, 5.25% (household liquid bleach)
- Mixer or Ultra-Turrax
- Plastic plate cover
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- Do not use water baths to thaw samples or reagents.
- Unused wells must be stored at 2-8 °C in the sealed foil pouch and used in the frame provided.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X Wash Buffer, warm to 37°C until the

crystals are completely dissolved.

- Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the mixed TMB Substrate. Do not expose the mixed TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Use a plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 60 minutes. Centrifuge at 2500 x g for 20 minutes.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Do not use heat-treated specimens.
3. Avoid disturbing the white buffy layer when collection serum/plasma sample.
4. Samples containing sodium azide should not be used in the assay.
5. Serum samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must store at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 30 mL of 20X Wash Buffer into 570 mL of distilled water to a final volume of 600 mL) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.

- TMB substrate: TMB substrate A and TMB substrate B should be mixed together in equal volumes up to 15 minutes before use.

- Standards:

A. Two vials of Standards are provided in this ELISA Kit. Reconstitute HAMA Standard with 2 mL of Diluent Buffer. This reconstitution produces a stock solution of 400 ng/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The HAMA standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles; aliquot if repeated use is expected.

B. Prepare a dilution series of HAMA Standards in the concentration range of 0 to 400 ng/mL by diluting the above Standards stock to the suggested concentration table below:

Standard tubes	Final HAMA (ng/mL)	Diluent Buffer (µL)	Standards (µL)
S1	400	0	1000 of 400 ng/ml standards stock
S2	200	500	500 of S1
S3	100	500	500 of S2
S4	50	500	500 of S3
S5	25	500	500 of S4
S6	12.5	500	500 of S5
S7	6.25	500	500 of S6
S0	0	500	0

Note: Dilutions for the standard must be made and applied to the plate immediately. S0 serves as background.

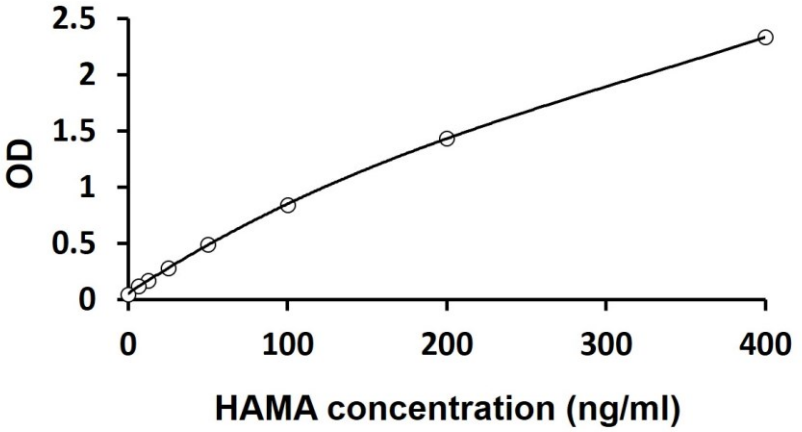
ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add 100 µL of the HRP Conjugate to the Antibody-coated microplate.
2. Add 50 µL of samples and Standards to the appropriate wells.
3. Cover and Incubate at RT for 1 hour on a microplate shaker.
4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µL of mixed TMB Substrate to each well, including the blank wells. Incubate for 15 minutes at room temperature in the dark.
6. Immediately Add 100 µL of Stop Solution to each well, including the blank wells. The color of the solution should change from blue to yellow.
7. Read the OD with a microplate reader at 450 nm immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 30 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the AMH ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. All O.D. values, are subtracted by the value of the standard 0 ng/mL.
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
7. HAMA expected value:
   Reference range study was conducted with 56 human serum and plasma samples from North American using this HAMA ELISA kit.
   The expected value range is from 0 to 79 ng/mL, and mean is 0.413 ng/mL.

QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of HAMA is 3 ng/mL.

Intra-assay and Inter-assay precision

The CV value of intra-assay was 7.6-11.8% and inter-assay precision was 6.5-11.2%