EAGLE BIOSCIENCES

Human Anti-Mouse Antibody (HAMA) ELISA Assay Kit

Catalog Number:
HAM31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 11.0 (31JUL24)

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INTENDED USE

The Eagle Biosciences Human Anti-Mouse Antibody (HAMA) ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of human anti-mouse IgG antibody (HAMA) levels in serum or plasma samples. The Eagle Biosciences Human Anti-Mouse Antibody (HAMA) ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at www.EagleBio.com or at 866-411-8023.

INTRODUCTION

Human Anti-Mouse Antibodies (HAMA) are human immunoglobulins (primarily IgG) that specifically recognize and bind to mouse-derived antibodies. These antibodies often develop in samples that have been exposed to murine monoclonal antibodies, either for diagnostic imaging, therapeutic purposes, or as part of clinical trials. Since many monoclonal antibodies used in research and clinical settings are originally derived from mice, the human immune system may recognize them as foreign, leading to the production of HAMA. The presence of HAMA can interfere with immunoassays, such as ELISAs or radioimmunoassays, by causing false positives or negatives. This is particularly important in diagnostic testing, where the accuracy of tumor markers, hormone levels, or cardiac biomarkers may be compromised. Clinically, HAMA formation is also a concern in patients receiving murine or chimeric therapeutic antibodies, as it can reduce drug efficacy, alter pharmacokinetics, or provoke hypersensitivity reactions.

In research applications, detecting and monitoring HAMA is crucial when using mouse-derived reagents in human samples. Researchers often screen for HAMA to avoid assay interference or unexpected immune responses in translational and preclinical studies. Anti-HAMA blocking agents or assay design modifications are commonly employed to mitigate its effects, ensuring data integrity when using murine antibodies in human-related research.

PRINCIPLE OF THE ASSAY

This assay is designed, developed, and produced for the quantitative measurement of HAMA in serum and plasma samples. The assay utilizes the two-site "sandwich" technique with two selected antibodies that bind to HAMA.

Assay standards, controls, and samples are directly added to wells of a microplate that is coated with murine IgG. After the first incubation period, the HAMA binds to the murine IgG on the wall of the microtiter well, and unbound proteins in each microtiter well are washed away. Then, a horseradish peroxidase (HRP) labeled murine IgG is added to each microtiter well, and a "sandwich" of "murine IgG HAMA—murine IgG" is formed. The unbound HRP-conjugated murine IgG is removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to HAMA on the wall of the microtiter well is directly proportional to the amount of HAMA in the sample. A standard curve is generated by plotting the absorbance versus the respective HAMA concentration for each standard on a point-to-point, cubic scale or 4-parameter curve fit. The concentration of HAMA in samples is determined directly from this curve.

PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- Practice good laboratory practices when handling kit reagents and specimens. This includes:
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
- Wear protective clothing and disposable gloves.

- Wash your hands thoroughly after performing the test.
- Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
- All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A calibrator curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (if applicable with this kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
- This kit contains 0.5 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of saker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.

- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

Specimen Collection & Storage

Only 50 µL of human serum or plasma is required for HAMA measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. In the case of serum, whole blood should be collected and must be allowed to clot for a minimum of 30 minutes at room temperature before the serum is separated by centrifugation (850-1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum or plasma samples should be stored at 2-8°C if the assay is to be performed within 72 hours. Otherwise, samples should be stored at -20°C or below until measurement. Avoid repeated (more than three times) freezing and thawing samples.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision single channel pipettes capable of delivering 25 µL, 50µL, 100 µL, and 1000 µL.
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
- Disposable plastic 100mL and 1000 mL bottle with caps.
- Distilled or deionized water.
- Aluminum foil.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

REAGENTS PROVIDED

1. **Microplate**

   Contents: One murine IgG coated 96-well (12x8) microplate in a resealable pouch with desiccant.

Format:	Ready to Use
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.

2. **HAMA Tracer Antibody**

Contents:	HRP-labeled anti-human IgG in a stabilized protein matrix
Format:	Concentrated
Volume:	0.6 mL/bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label
Preparation of working solution:	This reagent must be diluted with tracer antibody diluent prior to use.

3. **Tracer Antibody Diluent**

Contents:	For tracer antibody dilution
Format	Ready to Use
Volume	12 mL/bottle
Storage	2-8°C
Stability:	Stable until the expiry date printed on the label

4. **Assay Buffer**

Contents:	Phosphate buffered saline based assay buffer with bovine serum albumin added
Format:	Ready to Use
Volume:	30 mL/bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.

5. **ELISA Stop Solution**

Contents:	0.5 M sulfuric acid
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-25°C
Stability:	Stable until the expiry date printed on the label.

6. **HRP Substrate**

Contents:	Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.

7. **ELISA Wash Concentrate (30X)**

Contents:	Surfactant in a phosphate buffered saline with non-azide preservative.
Format:	Concentrated; Requires Preparation
Volume:	30 mL/bottle
Storage:	2-25°C
Stability	Stable until the expiry date printed on the label
Preparation	**Dilute 1:30**. The contents must be diluted with 870 mL distilled water and mixed well before use

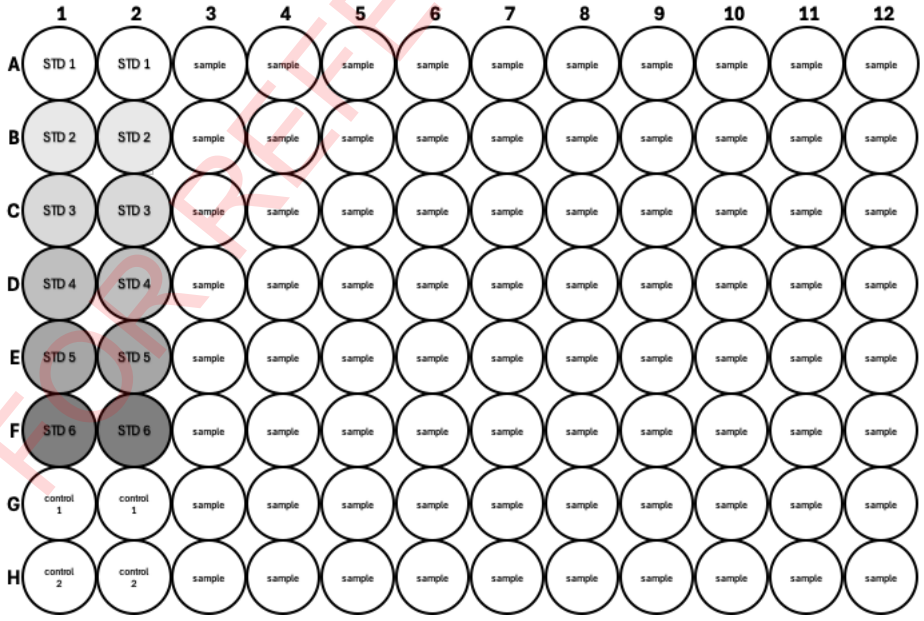
8. **HAMA Controls**

Contents:	Two controls in a liquid protein matrix with a non-azide based preservative. Refer to each vial for exact concentration
Format:	Ready to Use
Volume:	2 vials
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label

9. **HAMA Standards**

Contents:	Five vials of standards in a liquid protein matrix with a non-azide based preservative. Refer to each vial for exact concentration
Format:	Ready to Use
Volume:	5 vials
Storage:	2-8°C
Stability:	Stable until expiry date printed on the label

RECOMMENDED ASSAY LAYOUT*



**Layout subject to change based on standard and control quantities*

ASSAY PROCEDURE

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Place a sufficient number of microplate wells in a holder to run standards, controls, and samples in duplicate
2. **Add 25 µl** of standards, controls and samples into the designated microwells
3. **Add 100 µL** of Assay Buffer into each microwell.
4. Cover the plate with plate sealer and aluminum foil. **Incubate at room temperature (20-25°C) for 60 minutes.**
5. Remove plate sealer and aspirate contents of each well **5 times** by dispensing **350 µL** of prepared wash solution into each well, and then completely aspirate contents. Alternatively, an automated microplate washer can be used.
6. Prepare the HAMA working solution by 1:21 fold dilution of the HAMA Tracer Antibody with the Tracer Antibody Diluent. For each strip, it is required to mix 1 mL of the Diluent with 50 µL of the Tracer Antibody in a clean test tube. Discard remaining working solution after use.
7. **Add 100 µL** of HAMA Working Solution . Mix gently by tapping the plate.
8. Cover the plate with plate sealer and aluminum foil. **Incubate at room temperature (20-25°C) for 30 minutes.**
9. Remove plate sealer and aspirate contents of each well **5 times** by dispensing **350 µL** of prepared wash solution into each well, and then completely aspirate contents. Alternatively, an automated microplate washer can be used.
10. **Add 100 µL** of ELISA HRP Substrate into each of the wells. Mix gently by tapping the plate.
11. Cover the plate with plate sealer and aluminum foil. **Incubate at room temperature (20-25°C) for 20 minutes.**
12. Remove the aluminum foil and plate sealer. **Add 100 µL** of ELISA Stop Solution into each of the wells. Mix by gently tapping the plate.
13. Read the absorbance at **450/(595, 620 or 630)nm** within **10 minutes** with a microplate reader.

CALCULATIONS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the standard 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all other calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results. We recommend using Quadratic curve fit
4. The HAMA concentrations for the unknown samples are read directly from the standard curve using their respective corrected absorbance.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known HAMA levels. We recommend that all assays include the laboratory's own controls.

LIMITATIONS RELATED TO PROCEDURE

- For samples higher than level 5 standard, it is recommended to measure samples diluted with Assay Buffer at 1:10, 1:100, etc. for a more accurate report.
- Keep light sensitive reagents in the original amber bottle
- Bacterial or fungal contamination of serum specimens or reagents may cause erroneous results.
- Water deionized with polyester resins may inactivate the HRP enzyme.

TYPICAL DATA

Expected Values

One hundred seventy normal adult sera were measured with this HAMA ELISA. One hundred sixty sera showed the OD reading very close to the zero calibrator. The 99% confidence normal cut-off is 25 ng/mL.

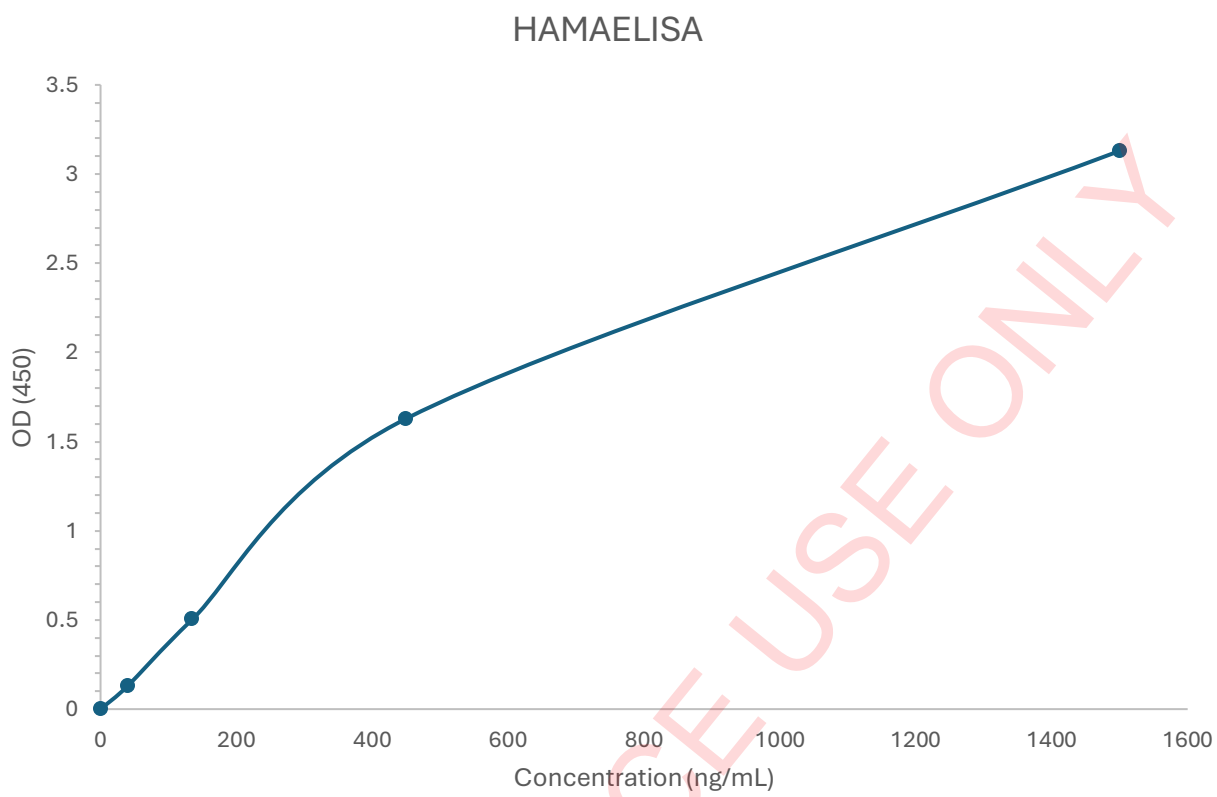
It is highly recommended that each laboratory establish it's own normal cut-off value.

One positive sample with HAMA level of 64 ng/mL was further tested with dilution of this sample in 1:2, 1:4, and 1:8. A linear HAMA dilution result was observed and indicated HAMA specific activity of this sample.

TYPICAL CALIBRATOR CURVE

A typical absorbance data and the resulting calibration curve from this HAMA ELISA are represented.
Note: this curve should not be used in lieu of the standard curve run with each assay

WELL ID	Reading Absorbance (450/620 nm)			Concentration (ng/mL)
	Readings	Average	Corrected	
Standard Level 1: 0 ng/mL	0.051	0.053	0.00	
	0.054			
Standard Level 2: 40 ng/mL	0.182	0.183	0.130	
	0.184			
Standard Level 3: 135 ng/mL	0.561	0.556	0.503	
	0.552			
Standard Level 4: 450 ng/mL	1.737	1.682	1.629	
	1.627			
Standard Level 5: 1500 ng/mL	3.230	3.183	3.130	
	3.136			
Control 1	0.284	0.296	0.243	64.16
	0.309			
Control 2	1.166	1.138	1.085	285.29
	1.109			



PERFORMANCE AND CHARACTERISTICS

Sensitivity

The sensitivity of the HAMA ELISA as determined by the 95% confidence limit on 20 duplicate determination of zero calibrator is approximately 2 ng/mL

Hook Effect

This assay has showed that it did not have any high dose "hook" effect up to 1,000,000 ng/mL

Reproducibility and precision

The intra-assay precision is validated by measuring one control sample in a single assay with 8 replicate determinations. The inter-assay precision is validated by measuring one control sample in duplicate in 6 individual assays. The results are as follows.

	Intra-Assay	Inter-Assay
Sample	**1**	**2**
Mean (ng/mL)	51.66	52.12
CV (%)	5.1	5.8

Linearity

Two (2) samples were diluted with assay buffer and tested. The results are as follows:

Samples	Observed (ng/mL)	Recovery (%)
Sample A	88.51	-
50%	44.98	101
25%	22.85	103
12.5%	37.15	113
Sample B	298.12	-
50%	141.93	95
25%	66.78	90
12.5%	37.15	100

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WARRANTY INFORMATION

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