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.

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INTENDED USE

This Eagle Biosciences Human Anti-Mouse Antibody ELISA Assay Kit is produced for the quantitative determination of human anti-mouse IgG antibody (HAMA) levels in serum or plasma samples. It detects both HAMA-IgG and HAMA-IgM subtypes. This kit is for research use only.

SUMMARY OF PHYSIOLOGY

Clinically, mouse monoclonal antibodies (IgG) and their fragments are used in vivo diagnosis procedure (radionuclides) and treatment for patients with various diseases. In patients, even a single dose injection of murine monoclonal IgG may induce immune response directed against this foreign protein (immunogen). In the circulation, the presence of human antibody against murine IgG would bind to the injected murine IgG and, therefore, diminish the efficacy of either in-vivo diagnosis or treatment. Especially, the HAMA would increase the risk of anaphylactic complications to subsequent administration of the murine IgG based therapy.

The presence of HAMA in patient serum or plasma specimens causes both false positive and false negative immunoassay test results depending on assay principles and monoclonal antibodies used in the assay system.

This HAMA ELISA is a ready-to-use test kit with well-breakable microtiter plate and simple test procedures. It also provides a wide measurement range without high dose "hook" effect.

ASSAY PRINCIPLE

The Human Anti-Mouse Antibody ELISA 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 calibrators, 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 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 calibration curve is generated by plotting the absorbance versus the respective HAMA concentration for each calibrator on point-to-point, cubical scales or 4 parameter curve fit. The concentration of HAMA in test samples is determined directly from this calibration curve.

REAGENTS

The Human Anti-Mouse Antibody ELISA test kit must be stored at 2 — 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

1. Murine IgG Coated Microplate
Microplate coated with murine IgG
Qty: 1 x 96 well microplate
Storage: 2 — 8°C
Preparation: Ready to Use

2. HAMA Tracer Antibody
HRP-labeled anti-human IgG in a stabilized protein in matrix.
Qty: 1 x 0.6 mL
Storage: 2 — 8°C
Preparation: This reagent must be diluted with tracer antibody diluent prior to use.

3. Tracer Antibody Diluent
For tracer antibody dilution.
Qty: 1 x 12 mL
Storage: 2 — 8°C
Preparation: Ready to Use

4. Assay Buffer
For tracer antibody dilution.
Qty: 1 x 30 mL
Storage: 2 — 8°C
Preparation: Ready to Use

5. ELISA Wash Concentrate
Surfactant in a phosphate buffered saline with non-azide preservative.
Qty: 1 x 30 mL
Storage: 2 — 25°C
Preparation: 30x Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

6. ELISA HRP Substrate
Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 12 mL
Storage: 2 — 8°C
Preparation: Ready to Use

7. ELISA Stop Solution
0.5 M sulfuric acid.
Qty: 1 x 12 mL
Storage: 2 — 25°C
Preparation: Ready to Use

7. HAMA Calibrators Levels 1 to 5

HAMA in a liquid protein matrix with non-azide based preservative.
Qty: 5 x Vials
Storage: 2 — 8°C
Preparation: Ready to Use

8. HAMA Controls

HAMA in a liquid protein matrix with a non-azide based preservative. Refer to each vial for exact concentration.
Qty: 2 x Vials
Storage: 2 — 8°C
Preparation: Ready to Use

SAFETY PRECAUTIONS

The reagents are for research use only. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

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

SPECIMEN COLLECTION

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 - 8ºC if the assay is to be performed within 72 hours. Otherwise, patient samples should be stored at - 20ºC or below until measurement. Avoid repeated (more than three times) freezing and thawing of specimen.

ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature (20-25 ºC). Reagents from different kit lot numbers should not be combined or interchanged.
(2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.

2. Assay Procedure

(1) Place a sufficient number of Murine IgG Coated microwell strips in a holder to run calibrators, controls, and samples in duplicate.
(2) Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
C	Calibrator Level 2	Control 1	SAMPLE 3
D	Calibrator Level 2	Control 1	SAMPLE 3
E	Calibrator Level 3	Control 2	SAMPLE 4
F	Calibrator Level 3	Control 2	SAMPLE 4
G	Calibrator Level 4	SAMPLE 1	SAMPLE 5
H	Calibrator Level 4	SAMPLE 1	SAMPLE 5

(3) Add 25 µL of calibrators, controls, and samples into the designated microwells.
(4) Add 100 µL of assay buffer into each microwell.
(5) Cover the plate with one plate sealer and aluminum foil. Incubate at room temperature for 60 minutes.
(6) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution into each well, then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(7) Prepare the antibody working solution by 1:21 fold dilution of the antibody with the tracer Antibody Diluent. For each strip, it is required to mix 1 mL of the tracer antibody diluent with 50 µL of the tracer antibody in a clean test tube.

Note: This antibody working solution should be freshly prepared.

(8) Add 100 µL of antibody working solution to each well. Mix by gently tapping the plate.
(9) Cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25 ºC) for 30 minutes.
(10) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
(11) Add 100 µL of ELISA HRP Substrate into each of the wells. Mix by gently tapping the plate.
(12) Cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25 ºC) for 20 minutes.
(13) 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.
(14) Read the absorbance at 450 / (595, 620, 630) nm within 10 minutes with a microplate reader.

PROCEDURAL NOTES

1. It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. For samples with higher than a level 5 calibrator, it is recommended to measure diluted the specimen with assay buffer at 1:10, 1:100, etc. for a more accurate report.
3. Keep light-sensitive reagents in the original amber bottles.
4. Store any unused murine IgG coated strips in the foil Ziploc bag with desiccant to protect from moisture.
5. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
6. Incubation times or temperatures other than those stated in this insert may affect the results.
7. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

INTERPRETATION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the level 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-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 controls and samples are read directly from the calibration curve using their respective corrected absorbance.

LIMITATIONS OF THE PROCEDURE

1. Since there is no Gold Standard concentration or international standard available for HAMA measurement, the values of assay calibrators were established and validated by 3rd party testing. Results obtained with different assay methods or kits cannot be used interchangeably.
2. For unknown sample values read directly from the assay that are greater than 1500 ng/mL, it is recommended to measure a further diluted sample for a more accurate measurement.
3. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

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 HAMA controls in addition to those provided with this kit.

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 recommend that each laboratory establish its own normal cut off level.

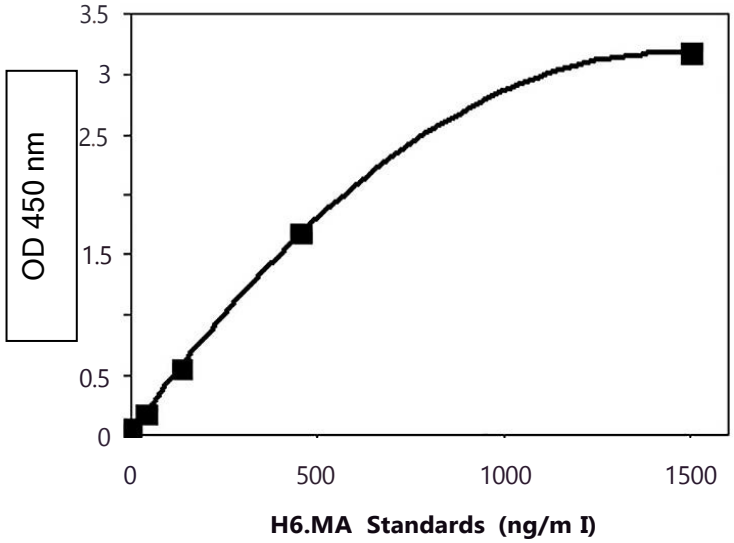
One positive sample with HAMA level of 64 ng/ml was further tested with dilution of this sample in 1:2, 1:4 and 1:8. A linear HAMA dilution result was observed and indicated HAMA specific activity of this sample.

EXAMPLE DATA

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 calibration curve run with each assay.

Well ID	Reading Absorbance (450 nm)			Concentration (ng/ml)
	Readings	Average	Corrected	
Calibrator Level1:0 ng/ml	0.051 0.054	0.053	0.00	
Calibrator Level2:40 ng/ml	0.182 0.184	0.183	0.130	
Calibrator Level3:135 ng/ml	0.561 0.552	0.556	0.503	
Calibrator Level4:450 ng/ml	1.737 1.627	1.682	1.629	
Calibrator Level 5:1500 ng/ml	3.230 3.136	3.183	3.130	
Control1	0.284 0.309	0.296	0.243	64.16
Control2	1.166 1.109	1.138	1.085	285.29



PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of this HAMA ELISA as determined by the 95% confidence limit on 20 duplicate determination of zero calibrator is about 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 was validated by measuring one control sample in a single assay with eight-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**	**1**
Mean (ng/mL)	51.66	52.12
CV (%)	5.1	5.8

Linearity

Two human serum samples were diluted with assay buffer and assayed. 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

Short Assay Procedure

1. Add 25 µL of the calibrators, controls, and samples into the designated microwells.
2. Add 100 µL of the assay buffer to each well.
3. Mix, cover, and incubate at room temperature (20-25ºC) for 60 minutes.
4. Wash each well five times.
5. Add 100 µL of the working tracer antibody to each well
6. Cover and incubate at room temperature (20-25ºC) for 30 minutes.
7. Wash each well five times
8. Add 100 µL of substrate to each well.
9. Cover and incubate at room temperature (20-25ºC) for 20 minutes.
10. Add 100 µL of the stop solution to each well.
11. Read the absorbance at 450 / (620, 630, or 650) nm.

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