HAMA (Human) ELISA Kit

Catalog Number KA1010

96 assays

Version: 05

Intended for research use only

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Introduction

Intended Use

This HAMA (Human) ELISA is to be used for the quantitative determination of human anti-mouse antibody (HAMA) in serum. It is useful for the prognosis, monitoring and follow-up patients treated with mouse IgG. This kit is intended for LABORTAROY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

Background

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. The HAMA assay measures HAMA across a wide dynamic range and it is not necessary to predilute human serum samples before the HAMA ELISA testing

Principle of the Assay

This assay applies an enzyme-linked immunosorbent assay (ELISA) technique. A purified mouse IgG has been pre-coated onto the wells of microplate. Standard (rabbit anti-mouse IgG) and specimens are added into the appropriate wells simultaneously with a HRP-labelled mouse IgG, and incubated. The Human Anti-Mouse Antibody (HAMA), if present, will bind with the mouse IgG which coated on the wells and HRP-labelled mouse IgG as well, forming a "Sandwich". Following a wash to remove any unbound substance, a substrate solution is added. The developed colour is directly proportional to the anti-mouse antibody concentration in the specimen and Standard samples.

This ELISA Kit includes a Calibration Diluent for preparing a serial 2-fold dilution of Standard series. It allows the operator to produce a standard curve with Optical Density (O.D) versus Human Anti-Mouse Antibody concentration (ng/mL). The concentration of Human Anti-Mouse Antibody in the sample is then determined by comparing the O.D. of the sample to the standard curve.

General Information

Materials Supplied

List of component

Component	State	Amount
HAMA Microtiter plate	Pre-coated with mouse IgG	96 wells
HRP Conjugate	HRP conjugate to Mouse IgG	12 mL
HAMA Standard	Rabbit anti Mouse IgG antibody (800 ng/vial) in a buffered protein base with Preservative, lyophilized.	2 vials
Calibrator diluent	Animal serum with buffer and preservative	25 mL
Wash Buffer (20x)	20-fold concentrated solution of buffered surfactant	60 mL
Substrate A	Buffered solution with H_2O_2	10 mL
Substrate B	Buffered solution with TMB.	10 mL
Stop solution	2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!	15 mL

Storage Instruction

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

Materials Required but Not Supplied

- ✓ Single or multi-channel precision pipettes with disposable tips: 10-100µl and 50-200µl for running the assay.
- ✓ Pipettes: 1 ml, 5 ml 10 ml, and 25 ml for reagent preparation.
- ✓ Multi-channel pipette reservoir or equivalent reagent container.
- ✓ Test tubes and racks.
- ✓ Polypropylene tubes or containers (25 ml).
- ✓ Microtiter plate reader (450 nm ± 2nm)
- ✓ Automatic microtiter plate washer or squirt bottle.
- ✓ Sodium hypochlorite solution, 5.25% (household liquid bleach).
- ✓ Deionized or distilled water.
- ✓ Plastic plate cover.
- ✓ Disposable gloves.
- ✓ Absorbent paper.

Precautions for Use

✓   Precautions

- Do not substitute reagents from one package lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.

- Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.

- Do not use reagents beyond their expiration date.

- Use only deionized or distilled water to dilute reagents.

- Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.

- Use fresh disposable pipette tips for each transfer to avoid contamination.

- Do not mix acid and sodium hypochlorite solutions.

- Human serum should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

- All samples should be disposed of in a manner that will inactivate human viruses.
  Solid Waste: Autoclave 60 min. at 121°C.
  Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

- Substrate Solution is easily contaminated. If bluish prior to use, do not use.

- Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.

- If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

✓   Limitations of the procedure

- FOR LABORATORY RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- The kit should not be used beyond the expiration date on the reagent label.

- If samples generate values higher than the highest standard, dilute the samples with the Calibrator Diluent and repeat the assay.

- We take great care to ensure that our products are suitable for use with all validated sample types, as designated in the product insert. However, it is possible that in some cases, high levels of interfering factors may cause unusual results.

- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and reagent age can cause variation in result.

- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of ligands in samples. However, until the factors have been tested, the possibility of interference cannot be excluded.
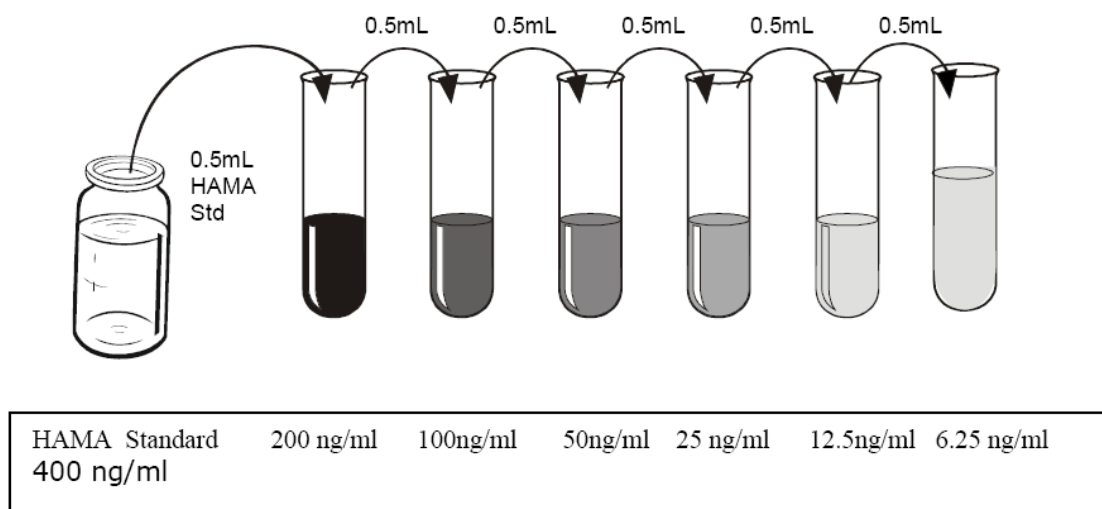
Assay Protocol

Reagent Preparation

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

✓ Wash Buffer (1X): Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

✓ Substrate Solution: Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (ml)	Substrate B (ml)	Substrate Solution (ml)
2 strips (16 wells)	2.0	2.0	4.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	7.0	7.0	14.0
12 strips (96 wells)	8.0	8.0	16.0

✓ HAMA Standard:

1. Two vials of Standard are provided in this ELISA Kit. Reconstitute HAMA Standard with 2.0 mL of Calibrator Diluent. 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.

2. Use the above stock solution to produce a serial 2-fold dilution series, as described below, within the range of this assay (6.25 to 400 ng/mL) as illustrated. Add 0.5 mL of the Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted HAMA stock solution will serve as the high standard (400 ng/mL) and the Calibrator Diluent will serve as the zero standard (0 ng/mL).

0.5mL   0.5mL   0.5mL   0.5mL   0.5mL

0.5mL
HAMA
Std

| HAMA Standard 400 ng/ml | 200 ng/ml | 100ng/ml | 50ng/ml | 25 ng/ml | 12.5ng/ml | 6.25 ng/ml |

Sample Preparation

Serum: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.

✓ Avoid grossly hemolytic, lipidic or turbid samples.

✓ 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.

✓ When performing the assay slowly bring samples to room temperature.

✓ It is recommended that all samples be assayed in duplicate.

✓ DO NOT USE HEAT-TREATED SPECIMENS.

Assay Procedure

1. Prepare Wash Buffer and HAMA Standards before starting assay procedure (see Preparation of Reagents). It is recommended that the plate layout provided be used as a reference for adding Standards and Samples to the Microtiter Plate.

2. Add 100 µL of HRP conjugate to the appropriate wells of antibody pre-coated Microtiter Plate.

3. Add 50 µL of Standard or Specimen to the appropriate wells. Mix well. Cover and Incubate for 1 hour at room temperature.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

✓ Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a total of FIVE washes. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the

plate to assure that all strips remain securely in frame.

✓ Automated Washing: Aspirate all wells, then wash plates FIVE times using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

5. Add 100 µL Substrate Solution into each well. Cover and Incubate for 15 minutes at room temperature.

6. Add 100 µL Stop Solution to each well. Mix by gently tapping the plate.

7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

Data Analysis

Calculation of Results

The standard curve is used to determine the amount of Human Anti-Mouse Antibody (HAMA) in an unknown sample. Create a standard curve by using a computer software or generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding HAMA concentration (ng/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values, are subtracted by the value of the zero standard (0 ng/mL) or (S1) before result interpretation. Construct the standard curve using graph paper or compute software.

2. To determine the amount of Human Anti-Mouse Antibody (HAMA) in each sample, locate the O.D. value on the Y-axis first, and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding Human Anti-Mouse Antibody (HAMA) concentration. If samples generate values higher than the highest standard, dilute the samples and repeat the assay, the concentration reading from the standard curve must be multiplied by the dilution factor.

✓ Typical Data

Results of a typical standard run of a HAMA ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. In the laboratory the Standard Curve should be established by each assay.

- Example

Standard (ng/mL)	Mean	Zero Standard Subtracted (Std.) – (S1)
0	0.044	0
6.25	0.120	0.076
12.5	0.167	0.123
25.0	0.277	0.233
50.0	0.491	0.447
100.0	0.842	0.798
200.0	1.432	1.390
400.0	2.332	2.290
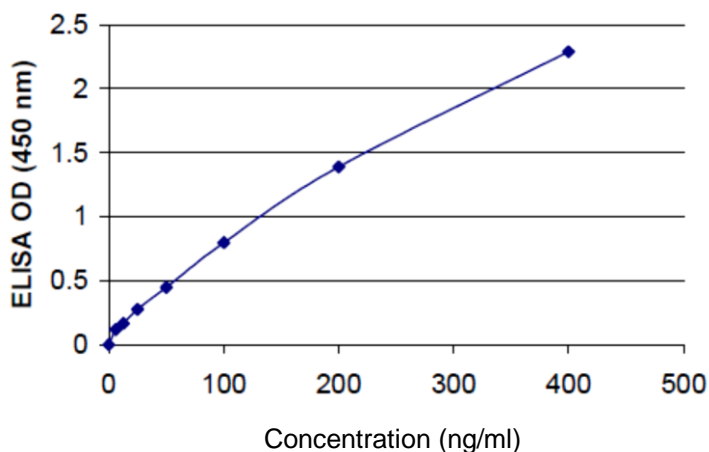
Performance Characteristics

✓ Expected Values:

Reference range study was conducted in 56 human serum and plasma samples from North America area using HAMA ELISA Kit. The following results were obtained:

Range: 0 - 79 ng/ml
Mean: 0.413 ng/ml

HAMA is not detectable or at very low concentration in most serum and plasma samples. Among the 56 tested samples, only two samples have HAMA reactivity level higher than 25 ng/ml.

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

✓ Intra-assay precision

Three different testing samples of known concentration were assayed twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/mL)	68.5	143.1	316.3
Standard Deviation (ng/mL)	8.1	10.9	24.2
Coefficient of Variation (%)	11.8	7.6	7.7

✓ Inter-assay precision

Three different samples of known concentration were assayed in 20 different separate assays to assess inter-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/mL)	66.8	155.9	339.1
Standard Deviation (ng/mL)	7.5	11.2	21.9
Coefficient of Variation (%)	11.2	7.2	6.5

✓ Hook Effect and Sensitivity

This kit didn't show any hook effect with a sample at 1 mg/ml of antibody.

The minimum detectable dose of HAMA was determined by adding two standard deviations to the mean optical density value of 10 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of HAMA is 3 ng/mL.

Resources

Reference

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Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1 0 ng/ml	S5 50 ng/ml	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
B	S1 0 ng/ml	S5 50 ng/ml	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
C	S2 6.25 g/ml	S6 100 ng/ml	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
D	S2 6.25 ng/m	S6 100 ng/ml	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
E	S3 12.5 g/ml	S7 200 ng/ml	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
F	S3 12.5 g/ml	S7 200 ng/ml	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
G	S4 25 ng/ml	S8 400 ng/ml	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
H	S4 25 ng/ml	S8 400 ng/ml	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample