BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit

Human Anti-Mouse Ig (HAMA)

Cat. No. 438307

ELISA Kit for Accurate Quantitation of HAMA from Serum and Plasma Samples

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biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

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Introduction:

Human Anti-Mouse Antibodies (HAMA) are antibodies found in human serum which have the ability to bind to mouse immunoglobulin (Ig). Pre-existing HAMA activity has been detected in approximately 5-10% of normal populations, which is believed to be caused by exposure to mice or mouse agents. Therapeutic use of mouse monoclonal antibodies or products derived from mouse monoclonal antibodies are becoming the most common cause of HAMA activities in patient serum. There is an increasing need to determine the levels of HAMA in human serum samples in therapeutic, assay development and diagnostic areas. For example, HAMA generated in response to injected monoclonal antibodies not only can bind and reduce the efficacy of the therapeutics, but also can cause complications to subsequent administration of mouse monoclonal antibodies. In addition, HAMA, due to its nature to bind mouse IgG, tends to cause false positive results in sandwich ELISAs and false negative results in competitive immunoassays utilizing mouse monoclonal antibodies. It is therefore important to measure serum samples for HAMA in cases of unexpected immunoassay results.

The BioLegend LEGEND MAX™ Human Anti-Mouse Ig (HAMA) ELISA kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a capture antibody.

This kit is specifically designed for the accurate quantitation of HAMA from serum and plasma samples (EDTA and heparin only). It is analytically validated with ready-to-use reagents.

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Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Mouse Ig Pre-coated 96-well Strip Microplate	1 plate		79394
Mouse Ig Conjugate	1 bottle	12 mL	79396
HAMA Standard	1 vial	lyophilized	79397
HAMA Quality Control 1	1 vial	lyophilized	79398
HAMA Quality Control 2	1 vial	lyophilized	79399
Matrix A	1 vial	lyophilized	78303
Assay Buffer A	1 bottle	25 mL	78232
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution C	1 bottle	12 mL	78114
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	1 pack		78101

Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate wells	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The reconstituted standard stock solution, QCs and Matrix A can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Quality Controls	
Matrix A	
Mouse Ig Conjugate	Store opened reagents between 2°C and 8°C and use within one month.
Assay Buffer A	
Wash Buffer (20X)	
Substrate Solution C	
Stop Solution	

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/support/#msds).
2. Substrate Solution C is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at ≤ -70°C. Avoid repeated freeze-thaw

cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Do not use citrate. Centrifuge for 15 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -70° C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use. Do not use sodium azide in any solutions as it inhibits the activity of the horseradish peroxidase enzyme, greatly reducing the signal. Standards should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized HAMA Standard by adding the volume of Assay Buffer A to make the 1,000 ng/mL standard stock solution (Refer toLEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 10 minutes, then briefly vortex to mix completely.
3. Reconstitute of the lyophilized Matrix A by dispensing 3 mL of deionized water into the vial. Allow the reconstituted Matrix A to sit at room temperature for 15 minutes, then briefly vortex to mix completely.
4. Reconstitute the lyophilized Quality controls (QCs) by adding the volume of Assay Buffer A described on the vial labels. Allow the reconstituted QCs to sit at room temperature for 10 minutes, then briefly vortex to mix completely.
5. In general, serum or plasma samples are assayed without dilution. If the measured sample value is above 250 ng/mL, then the sample should be diluted with reconstituted Matrix A and analyzed again.

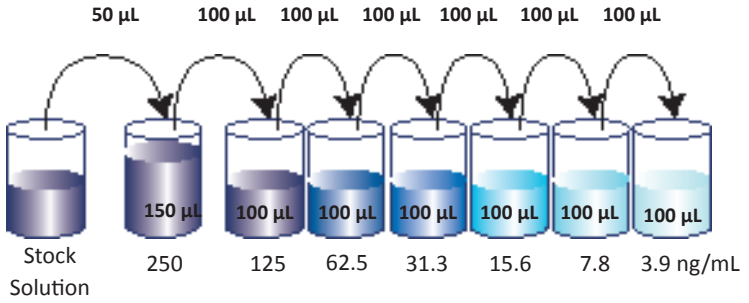
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 200 µL of the 250 ng/mL top standard by diluting 50 µL of the standard stock solution in 150 µL Assay Buffer A. Perform six two-fold serial dilutions of the 250 ng/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the HAMA standard concentrations in the tubes are 250 ng/mL, 125 ng/mL, 62.5 ng/mL, 31.3 ng/mL, 15.6 ng/mL, 7.8 ng/mL, and 3.9 ng/mL, respectively. Assay Buffer A serves as the zero standard (0 ng/mL).



4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.

5. Add 50 µL of Mouse Ig Conjugate to each well.

6. Add 25 µL of reconstituted Matrix A to the standard and QC wells and 25 µL of Assay Buffer A to the sample wells.

7. Add 25 µL of standard to the standard wells and 25 µL of QCs to the QC wells. Add 25 µL of samples to the sample wells.

8. Seal the plate with a plate sealer provided in the kit and then incubate at room temperature for 2 hours while shaking at 200 rpm.

9. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash

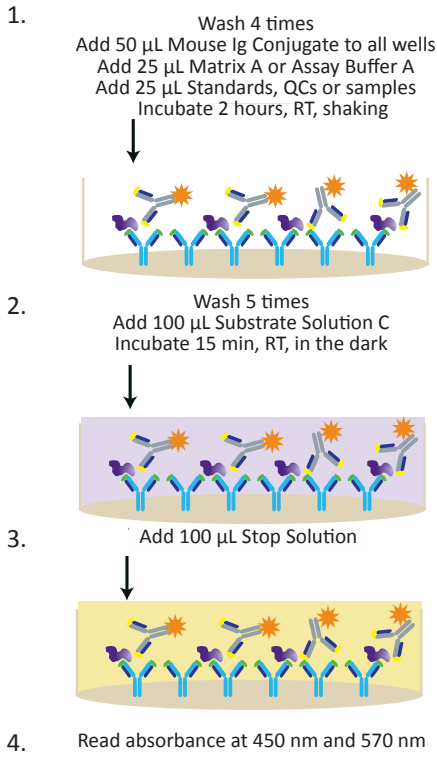
Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

10. Add 100 µL of Substrate Solution C to each well and incubate for 15 minutes in the dark. Wells containing HAMA should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

11. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

12. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary



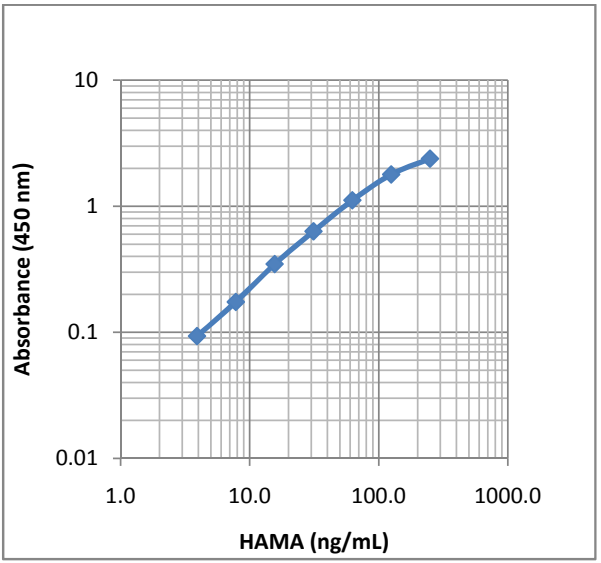
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with HAMA concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown HAMA concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the HAMA concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the quantifiable portion of the standard curve, the test sample needs to be further diluted or concentrated accordingly and re-analyzed.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



QC Ranges:

For the QC values, refer to the included QC vial labels.

Performance Characteristics:

Specificity: This kit is specifically designed to detect anti-mouse IgG including different IgG subtypes.

Sensitivity: The minimum detectable concentration of HAMA is 1 ng/mL.

Recovery: Three levels (250, 62.5 and 15.6 ng/mL) of anti-mouse IgG were spiked into 5 human serum samples, and then analyzed using this kit. On average, 101.7 % of the spiked samples were recovered.

Linearity: Five human serum samples with high concentrations of HAMA were diluted with Matrix A to produce samples with values within the dynamic range and then assayed. The linearity of dilution ranged from 85.7-122.3%. On average, a 107.2% of linearity of dilution was observed.

Intra-Assay Precision: Two samples containing different HAMA concentrations were tested with 14 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	14	14
Mean Concentration (ng/mL)	19.9	88.2
Standard Deviation	0.24	2.0
%CV	1.0%	2.3%

Inter-Assay Precision: Two samples with different concentrations of HAMA were assayed in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (ng/mL)	18.9	89.2
Standard Deviation	1.5	5.3
%CV	7.9%	5.9%

Biological Samples:

Normal human serum and plasma (EDTA and heparin) samples (n = 84) were assayed for basal levels of HAMA. HAMA levels ranged from undetectable to 111.3 ng/mL, with a mean of 9.7 ng/mL and median of 4 ng/mL. Twelve samples (14.3%) contained HAMA levels above 10 ng/mL.

It is recommended that each laboratory establish its own reference values for positive samples.

Limitations of the Assay:

- The exact nature of HAMA is not completely understood. The test results are therefore subject to interpretation.
- Azide interferes with this assay. Therefore, no azide should be used in preparation of samples or in any end-user provided reagents used in the assay.
- Citrate plasma is not suitable for this assay.

Troubleshooting Guide:

Problem	Probable Cause	Solution
No or poor signal	Mouse Ig Conjugate or Substrate Solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

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Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, Mouse Ig Conjugate or Substrate Solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes

LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
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