

IMMUNOMEDICS[®], INC.

ImmustrIP[®] HAMA IgG

ELISA Test System for Human Antibodies to Mouse IgG

Catalog Number: 10016
For *In Vitro* Diagnostic Use

INTENDED USE

The ImmustrIP[®] HAMA IgG ELISA Test System is a direct enzyme-linked immunosorbent assay for the detection and semi-quantitation of human antibodies to mouse IgG (HAMA). The presence of human anti-mouse antibody (HAMA) has been associated with patients receiving injections of murine monoclonal antibody for diagnostic and/or therapeutic purposes.

SUMMARY AND EXPLANATION

The ImmustrIP[®] HAMA IgG ELISA Test System is a direct enzyme-linked immunosorbent assay for the detection and semi-quantitation of human humoral antibodies to mouse IgG (1).

Significant levels of human anti-mouse IgG antibody (HAMA) have been associated with patients receiving injections of murine monoclonal antibody (MAb) (2-6).

The use of radioimmunotherapy has significantly increased the number of patients with HAMA titers. Circulating levels of HAMA can complex the injected antibody and adversely affect the imaging and/or therapy that was intended (5). In addition, HAMA has been shown to significantly interfere with many commercial assays utilizing murine MAbs, resulting in both false-positive and false-negative results (7-12).

The ImmustrIP[®] HAMA IgG ELISA Test System may be performed manually or with existing microtiter equipment. Results, which are read at 450nm, are available in less than 1 hour. The test has been calibrated with baboon anti-mouse IgG serum and has a sensitivity of 37 ng/mL. Final patient values are reported as nanograms of precipitable antibody equivalents per mL.

It is recommended that baseline HAMA levels are determined prior to the initiation of therapy with murine-derived proteins.

PRINCIPLE OF THE PROCEDURE

The HAMA assay utilizes the ELISA technique for the detection of human anti-mouse antibody (HAMA).

Mouse IgG conjugated to horseradish peroxidase enzyme (conjugate) is added to the microwell which has been coated with mouse IgG whole molecule. Diluted test sample is then added and incubated. If antibody to the mouse IgG is present in the test sample, antigen-antibody complexes are formed. If antibody is not present in the test sample, the unbound conjugate will be removed in the subsequent washing step.

Enzyme substrate is then added to the microwell. If bound conjugate is present, the substrate will be reduced; the reduced end-product oxidizes the colorless chromogen resulting in a colored end-product. Acid is added to stop the reaction and fix the color.

The absorbance is measured with a microwell strip reader at a wavelength of 450nm. The absorbance of the solution is directly proportional to the amount of bound conjugate and, therefore, to the concentration of precipitable antibody present in the sample.

REAGENTS

Recommended storage and stability: Store at 2-8°C.

Reagents from kits with different lot numbers must not be interchanged.

Kit Components (96 tests), Product Code 10016:

- Twelve (12) Microwell strips (8 wells per strip) coated with mouse IgG, whole molecule.
- One vial Conjugate Concentrate: 10X concentrated enzyme conjugate in PBS-buffered protein solution containing gentamicin sulfate.
- One vial Specimen Diluent: Buffered human serum containing 0.005% gentamicin sulfate and 0.02% thimerosal.
- One Vial Calibrator: Baboon anti-mouse IgG serum containing 220ng of precipitable antibody equivalents per mL.
- One vial Positive Control: Baboon anti-mouse serum diluted with specimen diluent, containing 100-150ng of precipitable antibody equivalents per mL.
- One bottle TMB enzyme substrate.
- One bottle of 10X phosphate-buffered saline (PBS) containing polysorbate 20.
- One sample dilution plate.
- One microwell strip holder.

Instructions for preparation of reagents are provided in the section entitled PREPARATION OF REAGENTS.

MATERIALS REQUIRED BUT NOT PROVIDED

1. 1mL, 5mL, and 10mL serological pipettes.
2. 200 μ L adjustable single channel micropipette.
3. 50 μ L to 200 μ L adjustable multichannel micropipette.
4. 50 μ L to 200 μ L disposable pipette tips (recommended by multichannel micropipette manufacturer).
5. Multichannel micropipette reservoir.
6. Disposable 12 x 75 mm or 13 x 100mm glass tubes.
7. Disposable plastic 25mL and 50mL tubes with caps.

8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Plastic microwell strip cover or polyethylene film.
10. Aliquot mixer.
11. 37°C incubator.
12. Microwell strip reader capable of reading at 450nm.
13. Glass-distilled or deionized water.
14. 2N (normal) sulfuric acid.
15. 1 liter volumetric glass container.
16. Bleach for disinfectant and disposal of waste.

PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Source material from which reagents of human origin were derived were found nonreactive for HBsAg and HIV-1 when tested with licensed reagents. No known test method can offer complete assurance that products derived from blood will not transmit hepatitis or other infectious agents. Reagents derived from animal sera have not been tested. **Handle these reagents as if they are potentially infectious.**
3. Wear gloves while performing this assay.
4. Household bleach should be used to wipe up spills and should be added to waste material prior to disposal.
5. Do not mix reagents from different kit lots. The reagents in this test kit are formulated to be used as a kit for detection of HAMA. Dilution or adulteration of reagents other than directed will result in loss of sensitivity. Cross contamination of reagents and patient samples, or use of reagents other than those supplied in the kit may produce erroneous results.
6. Do not use kit components beyond the expiration date.
7. Do not reuse microtiter wells.
8. Store unused reagents at 2-8°C.
9. 2N Sulfuric Acid may cause severe irritation on contact with skin. 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.
10. Handle the TMB with care. Avoid contact with eyes, skin or clothing as TMB may cause irritation or an allergic skin reaction. The TMB is light sensitive. Keep vial **tightly** closed when not in use. Bring only the required amount to room temperature before use.

SPECIMEN COLLECTION

No special preparation of the individual is required prior to specimen collection. The whole-blood test specimen should be collected by accepted medical techniques. **The specimen must be allowed to clot for two hours at room temperature (15-30°C) before the serum is separated by centrifugation.**

The serum should be separated from the clot within eight hours of collection and transferred to a glass test tube. The test specimen can be centrifuged at 850-1500xg for 10 minutes at ambient temperature (15-30°C). Mechanical serum separating devices may be used. The resulting test specimen must be completely free of cellular debris. Once the serum has been separated from the clot, it may be stored at 2-8°C for up to 24 hours.

If the serum specimen cannot be tested within one day of collection, the specimen should be stored at -70°C until tested. Serum samples are stable at -70°C for at least three months. Freeze and thaw only once. Specimens may be thawed either at room temperature or for up to 5 minutes at 37°C

1. Serum is required for the HAMA assay. Plasma or anticoagulated specimens collected as plasma and then clotted should not be used.
2. Do not heat-inactivate serum specimens.

PREPARATION OF REAGENTS

1. Preparation of PBS-polysorbate 20

Dilute the 100 mL of 10X PBS polysorbate 20 concentrate with 900 mL of distilled or deionized water. Mix thoroughly to dissolve any crystals that may be present.

2. Preparation of Conjugate

Disposable glass or plastic ware must be used. The conjugate concentrate must be diluted with PBS-polysorbate (prepared above) just prior to use. Dilute an aliquot of the conjugate concentrate 1:10. The diluted conjugate should be prepared just prior to use and should be used within 30 minutes. **For 96 tests (12 microwell strips):** Add 1mL conjugate concentrate to 9mL PBS-polysorbate. A volume of 10mL is needed for 12 microwell strips.

3. Preparation of 2N Sulfuric Acid

For 96 tests (12 microwell strips): Add 0.5mL of concentrated (36N) sulfuric acid to 8.5 mL of glass distilled or deionized water. Always add the acid to the water. Never reverse the procedure. This volume is sufficient for 12 microwell strips.

4. Preparation of Test Samples

Prepare a 1:2 dilution of all patient samples with specimen diluent. Greater dilutions may be required for high positive samples.

5. Preparation of Calibrators

To semi-quantitate positive samples, a calibrator curve is necessary. The curve should be prepared from the calibrator supplied in the kit by diluting an aliquot with specimen diluent. Suggested calibrator dilutions are 1:2, 1:4, and 1:6.

6. Preparation of Positive Control Sample

Prepare a 1:2 dilution of the positive control sample supplied in the kit, with specimen diluent.

PROCEDURAL NOTES

1. Do not allow the wells to dry during the test. Drying of wells may result in falsely high absorbance values.
2. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
3. Incubation times or temperatures other than those stated in this insert may affect the results.
4. Avoid air bubbles in microtiter wells as this could result in lower binding efficiency.
5. Washing procedures different than stated may adversely affect the results.
6. All liquid reagents should be mixed thoroughly prior to use. Avoid foaming.

TEST PROCEDURE

1. Prepare PBS-polysorbate. Refer to PREPARATION OF REAGENTS.
2. Using disposable glass tubes, prepare 1 : 2, 1 : 4, and 1 : 6 dilutions of the HAMA calibrator in specimen diluent.
3. Calibrator dilutions are prepared using the HAMA calibrator (C1) as follows:

Calibr.	Antibody conc. ng/mL	HAMA Calibr.(μ L)	Vol. Specimen Diluent(μ L)	Test Dilution
(C1)	220	-	-	Undiluted
(C2)	110	200	200	1 : 2
(C3)	55	100	300	1 : 4
(C4)	37	100	500	1 : 6

NOTE:
Specimen diluent can be used as both a reagent control (RC) and a 0 ng/mL calibrator.

For quality control of the assay, a 1 : 2 dilution of the positive control (PC) sample is prepared using specimen diluent. The positive control sample should be assayed in duplicate each time the ImmuSTRIP[®] HAMA IgG assay is performed.

5. Remove an appropriate number of microwell strips from their protective pouches and place in a microwell strip holder.

NOTE: Each aluminum pouch contains 2 microwell strips that are sealed with desiccant to reduce moisture. If only 1 microwell strip is removed for an assay, reseal the pouch by folding and taping, leaving the desiccant inside. When stored at 2° to 8°C, resealed pouches will retain their reactivity. Pouches that have not been resealed or refrigerated after opening should be discarded.

7. Appropriately label all strips. All test samples, calibrators and the positive control sample should be run in duplicate.

7. TEST CONFIGURATION

ROW	STRIP 1	STRIP 2
A	BL	C4
B	RC	C4
C	C1	PC
D	C1	PC
E	C2	
F	C2	
G	C3	
H	C3	

8. Add 0.2 mL of diluted PBS-polysorbate to the blanking well (BL).

9. Add 0.1 mL of diluted conjugate to all wells to be used except the blanking well (BL).

10. Add 0.1 mL of specimen diluent to the reagent control well (RC).

11. Add 0.1 mL of each diluted calibrator, the diluted positive control (PC) and each diluted test sample to the appropriate wells on the strips.

12. Cover the microwell strip holder and incubate at 37°C for 30 minutes.

13. After the 30-minute 37°C incubation, discard the contents of the microwell strips and wash the wells 3 times with diluted PBS-polysorbate solution. The washing procedure must be thorough. Washing may be performed using an automatic microtitration washing device or manually as follows:

- Empty wells by vigorously shaking out contents; grip sides of strip holder firmly to prevent strips from falling out of the holder.
- Fill wells with diluted PBS-polysorbate using a wash bottle.
- Remove diluted PBS-polysorbate by shaking out contents.
- Repeat steps b and c twice.
- After the final wash, tap the plate holder firmly on a clean paper towel to remove excess diluted PBS-polysorbate.
- Proceed immediately to the next step.

14. Add 0.2 mL of substrate solution to all wells, including the blanking well (BL).

15. Place the strips in the dark at room temperature for 15 minutes.

16. Using a multichannel micropipette, forcibly eject 0.05 mL of 2N sulfuric acid into each well, including the blanking well (BL). It is important that the sulfuric acid is spread quickly and uniformly throughout the wells to completely inactivate the enzyme.

17. Set the microwell strip reader at a wavelength of 450 nm and blank the reader according to the manufacturer's instructions by using the blanking well (BL) containing substrate and acid.

18. Measure the color intensity in all microwells.

INTERPRETATION OF RESULTS

A. Control and Calibrators

1. Reagent Control

Record the absorbance value of the reagent control (RC) well. If the reagent control was run in duplicate, calculate the mean absorbance value. If equal to or greater than 0.14, the test should be repeated.

2. Calibrators

Calculate the mean absorbance value of the duplicate calibrators. For HAMA, the following values have been assigned.

C1	220 ng/mL
C2	110 ng/mL
C3	55 ng/mL
C4	37 ng/mL
RC	0 ng/mL

B. Preparation of the Calibrator Curve and Determination of HAMA Concentration (nanograms of precipitable antibody equivalents/mL) for the positive control and each test sample.

1. Calculation Method

Calculations can be made on a variety of calculators supplied with linear regression programs. Follow the manufacturer's instructions for use of these programs to establish the calibrator curve and the HAMA concentration (nanograms of precipitable antibody equivalents/mL) of the positive control and each test sample.

In order for an assay to be valid, the following criteria must be met:

- a. Slope 0.005 - 0.015
- b. Y-intercept ≤ 0.200
- c. Correlation coefficient (r^2) ≥ 0.950

NOTE: The positive control sample contains 100 - 150 ng of precipitable antibody equivalents per mL.

2. Graphic Method

- a. Plot the mean absorbance values for each calibrator on the ordinate against the corresponding nanograms of precipitable antibody equivalents/mL HAMA on the abscissa using linear graph paper. Refer to INTERPRETATION OF RESULTS (Calibrators) for assigned values expressed as ng/mL.
- b. Draw a straight line for the **best fit** of the plotted points.
- c. Locate the point corresponding to the mean absorbance value of the positive control and each test sample, and read off the corresponding ng/mL HAMA.
- d. Record HAMA concentration in ng/mL as determined above.

LIMITATIONS OF PROCEDURE

1. Heat-inactivated samples may result in false-negative results.
2. The presence of Rheumatoid Factor (RF) and/or heterophilic antibodies in the sample may interfere with the assay and could result in false-positive values (10, 11). If aberrant test results are obtained, it is recommended that the patient be tested for the presence of these interfering substances prior to therapy initiation. REFER TO SPECIFICITY/INTERFERING SUBSTANCES.
3. Lipemic serum samples may interfere with the assay and could result in false-positive values. REFER TO SPECIFICITY/INTERFERING SUBSTANCES.
4. Improper or insufficient washing at any stage of the procedure will result in either false-positive or false-negative results.
5. Adherence to the humidity, temperature and time periods for incubation is essential for accurate results.
6. Bacterial or fungal contamination of serum specimens or reagents, or cross contamination between reagents may cause erroneous results.
7. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

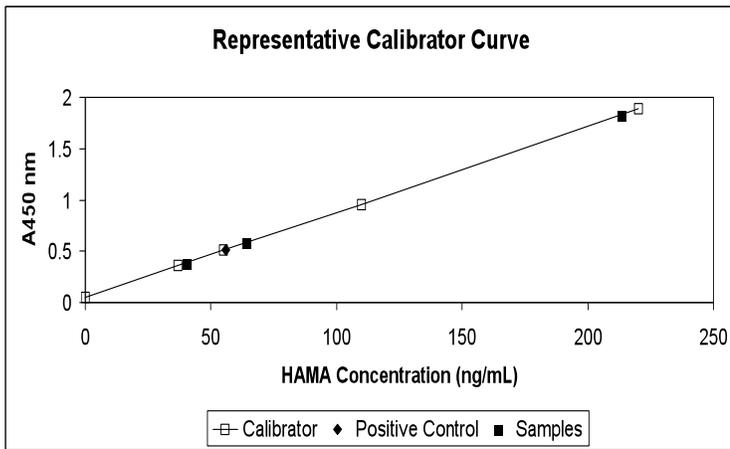
REPRESENTATIVE CALIBRATOR CURVE

A representative calibrator curve is shown in Table 1 and graphically presented. This curve cannot be used to derive test results. Each laboratory must prepare a Calibrator Curve for each group of microwell strips assayed (up to twelve).

Table 1

	Mean Absorbance Value	HAMA (ng/mL)
Calibrator 1	1.889	220
Calibrator 2	0.955	110
Calibrator 3	0.512	55
Calibrator 4	0.365	37
Reagent Control	0.048	0
Positive Control	0.517	56 (2) = 112
Sample 1	1.828	213 (2) = 426
Sample 2	0.582	64 (2) = 128
Sample 3	0.382	40 (2) = 80
Slope	0.008	
Y-Intercept	0.049	
Correlation	0.999	

NOTE: The assay requires an initial 1:2 dilution of the positive control and test samples. HAMA values extrapolated from the calibrator curve must be multiplied by 2 for the final HAMA concentration. Some test samples may require a higher dilution factor. The value as ng/mL must be multiplied by the dilution factor of the test sample.



SUMMARY OF PERFORMANCE CHARACTERISTICS

Reproducibility

a. Within run and between run assay reproducibility was evaluated by performing the ImmuSTRIP® HAMA IgG assay using low (50 ng/mL), medium (75 ng/mL) and high (200 ng/mL) control samples. The control samples were prepared by enriching normal human serum with purified HAMA.

Results: Between run CVs were calculated from assays performed on three differendays. Each control sample was assayed in triplicate; the mean of the triplicates was reported. The coefficients of variations (CVs) for within run values were ≤ 10 %, and between run CVs were < 5%. Please refer to the table below:

(a) Within Run and Between Run Assay Reproducibility

Run #	Low Control (50 ng/mL)			Medium Control (75ng/mL)			High Control (200 ng/mL)		
	ng/mL	SD	CV(%)	ng/mL	SD	CV(%)	ng/mL	SD	CV (%)
1	53.4	±5.2	9.7	76.3	±2.1	2.8	187	±5.9	3.2
2	56.0	±2.5	4.5	72.5	±0.5	0.7	193	± 18	9.2
3	51.4	±1.8	3.5	71.5	±3.2	4.5	193	± 19	10.0
Between Run Mean	53.6	±2.3	4.3	73	±2.5	3.4	191	±3.7	1.9

b. Lot to lot reproducibility was performed by assaying six prepared serum controls with three lots of ImmuSTRIP® HAMA IgG reagents. Six serum controls were prepared by enriching normal human serum with varying concentrations of purified HAMA. The range in HAMA concentration was 50 ng/mL to 400 ng/mL. The HAMA results were obtained from three calibrator curves prepared from the calibrator provided with each lot; all results are expressed as a mean of triplicate values. Please refer to the following table:

(b) Lot to Lot Reproducibility for 6 Prepared Serum Controls

Serum (ng/mL)	HAMA (ng/mL)			Avg	SD	CV (%)
	Lot 1627	Lot 1628	Lot 1629			
50	56.6	47.4	53.1	52.4	3.8	7.2
75	92	78.6	76.0	82.2	7.0	8.5
100	99	99.8	98.6	99.1	0.5	0.5
200	211	220	187	206	13.9	6.8
300	303	310	291	301	7.8	2.6
400	395	344	374	371	20.9	5.6

Sensitivity

The sensitivity of the ImmuSTRIP® HAMA IgG is 37 ng of precipitable antibody equivalents/mL. The lowest concentration prepared from the calibrator (C1) is equal to 37 ng precipitable antibody equivalents/mL and is the labeled sensitivity of the assay. The recommended dilution of test sample is 1:2, therefore, a negative HAMA result has been defined as ≤74 ng precipitable antibody/mL, [37 X 2 (dilution factor)].

Expected Values

The following table summarizes the distribution of HAMA values in an apparently healthy population.

Healthy Subjects	< 10 ng/mL	10-74 ng/mL	75-99 ng/mL	100-199ng/mL	200-299 ng/mL
300 Males	41.2	56.8	0.8	0.4	0.8
110 Females	49.1	50.9	0	0	0
104 Pregnant Females	35.6	60.5	1	1	2

The ImmuSTRIP® HAMA IgG assay defines a "negative" as ≤ 74 ng/mL. In this healthy population, 98% of the males, 100% of the non-pregnant females and 96.1% of the pregnant females were negative for HAMA. The presence of antibodies to mouse immunoglobulin in the serum of healthy individuals has been documented in several studies^{10, 11, 16}.

Specificity/Interfering Substances

(a) Rheumatoid Factor Samples

An evaluation of the specificity of ImmuSTRIP® HAMA IgG was performed by assaying 57 serum samples having various concentrations of rheumatoid factor (RF). RF are autoantibodies directed to the Fc portion of human and other mammalian IgG molecules. In this study, serum RF concentrations, expressed in International Units (IU), varied in the range from negative (normal) to 3000 IU (high). All positive samples were from patients diagnosed with rheumatoid arthritis. All samples were assayed in duplicate, the mean of the duplicate values was reported. The patient population from which these samples had been collected either had no previous exposure to mouse protein or such exposure was unknown. Twenty-one (36.8%) of the RF serum samples resulted in falsely elevated HAMA test results (> 74 ng/mL). There was no apparent correlation between the level of RF in the sample and the severity of interference with the ImmuSTRIP® HAMA IgG assay.

(b) The following table lists ImmuSTRIP® HAMA IgG ELISA test results on serum samples containing other potentially interfering substances.

# of Serum Samples Tested	Description of Interfering Substances	Amount of interfering Substance in Sample	ImmuSTRIP® HAMA IgG Test Result
38	Chemotherapeutic Serum Samples**	N/A*	37 Samples - Negative **1 Sample - Positive (85.3 ng/mL)
10	Hemolyzed Serum Samples	N/A*	10 Samples - Negative
10	Lipemic Serum Samples	Grossly Lipemic	9 samples - Negative ***1 sample - Positive (232 ng/mL)
2	Digoxin Serum Samples	1 - 2.7 ng/mL	2 Samples - Negative
10	Dilantin Serum Samples	5 - 10 µg/mL	10 Samples - Negative
2	Phenobarbital Serum Samples	22 - 30 µg/mL	2 Samples - Negative
3	Vancomycin Serum Samples	800.35 µg/mL	3 Samples - Negative
3	Theophylline Serum Samples	12.1 - 15.8 µg/mL	3 Samples - Negative
10	Protein Serum Samples	6.5 - 8.3 g/dl	10 Samples - Negative
5	Protein Serum Samples (Elevated)	8.9 - 9.5 g/dl	4 Samples - Negative ****1 Sample - Positive (100 ng/mL)
5	Protein Serum Samples (Low)	5.3 - 5.7 g/dl	5 Samples - Negative
9	Acetaminophen/Aspirin Serum Samples	N/A*	9 Samples - Negative

* N/A Not Applicable

**Included serum samples from patients undergoing chemotherapy with the following agents: 5FU, Leucovorin, Cytosine, Urokinase, Cisplatin, Taxol, Vinblastin, Carboplatinum, Levamisole, and Ondansetron. One sample tested slightly positive (85.3 ng/mL) with Immu-STRIP® HAMA IgG; this result remains unexplained.

***One sample from the grossly lipemic serum group tested positive (232 ng/mL) with the ImmuSTRIP® HAMA IgG assay. Please refer to LIMITATIONS OF PROCEDURE.

****One sample from the elevated serum protein group tested positive with the ImmuSTRIP® HAMA IgG assay. This sample was further analyzed for the presence of Rheumatoid Factor, (see section (a) above) and was strongly positive (4+) with an RF latex assay.

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