Datasheet for ABIN1305179

HAMA ELISA Kit

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Overview

Quantity:	96 tests
Target:	HAMA
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	2.0-1500 ng/mL
Minimum Detection Limit:	2 ng/mL
Application:	ELISA

Product Details

Purpose:	This ELISA (enzyme-linked immunosorbent assay) kit is produced for the quantitative determination of human anti-mouse IgG antibody (HAMA) levels in patient serum or plasma samples. It detects both HAMA-IgG and HAMA-IgM subtypes. The test might be used as an aid for detection of patients with positive HAMA that may affect prescribed diagnosis and treatment involving monoclonal murine IgG.
Brand:	ED™
Sample Type:	Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	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.
Components:	1. Murine IgG Coated Microplate

Product Details

	Two vials each containing 0.5ml of a different level of HAMA in a liquid protein matrix with a non-azide based preservative. Refer to vials for exact concentration range for each control. Both controls should be stored at 2-8 °C and are stable until the expiration date on the kit box.
Material not included:	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.

Target Details

Target:	HAMA
Abstract:	HAMA Products
Target Type:	Antibody
Background:	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.

Application Details

Sample Volume:	50 µL
Assay Time:	4 h
Plate:	Pre-coated

Application Details

Protocol:	Assay standards, controls and patient 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 standard curve is generated by plotting the absorbance versus the respective HAMA concentration for each standard on point-to-point, cubical scales or 4 parameter curve fit. The concentration of HAMA in test samples is determined directly from this standard curve.
Reagent Preparation:	(1) Prior to use allow all reagents to come to room temperature. 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.
Sample 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 - 8 C if the assay is to be performed within 72 hours. Otherwise, patient samples should be stored at - 20 °C or below until measurement. Avoid repeated (more than three times) freezing and thawing of specimen.
Assay Procedure:	(1) Place a sufficient number of murine IgG-coated microwell strips/wells in a holder to run HAMA standards, controls and unknown samples in duplicate. (2) Test Configuration (3) Add 25 µL of standards, controls and patient samples into the designated microwell. (4) Add 100 µL of assay buffer to each well (5) Cover the plate with one plate sealer and incubate plate at room temperature for 1 hour. (6) Prepare HAMA Tracer 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. (7) Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by

Application Details

	dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (8) Add 100 µL of above diluted HAMA Tracer Antibody working solution to each of the wells. (9) Cover the plate with the plate sealer and incubate plate at room temperature for 30 min. (10) Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (11) Add 100 µL of ELISA HRP Substrate into each of the wells. (12) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light. (13) Incubate plate at room temperature for 20 min. (14) Remove the aluminum foil and plate sealer. Add 100 µL of ELISA Stop Solution into each of the wells. Mix gently. (15) Read the absorbance at 450 nm within 10 minutes in a microplate reader. NOTE: to reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595 nm or 620 nm or 630 nm.
Calculation of Results:	1. Calculate the average absorbance for each pair of duplicate test results. 2. Subtract the average absorbance of the STD 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 standard levels on the ordinate against the standard 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. The HAMA concentrations for the controls and patient samples are read directly from the standard curve using their respective corrected absorbance.
Assay 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.
Restrictions:	For Research Use only

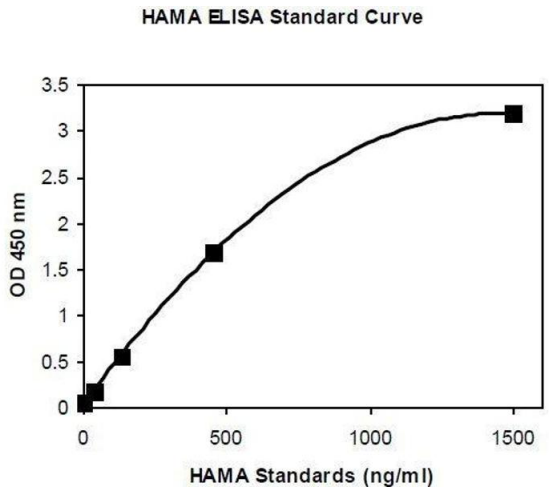
Handling

Precaution of Use:	The reagents must be used in a professional laboratory environment and are for research use only. Source material (e.g. highly purified bovine serum albumin) of bovine serum 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 are potentially infectious. Avoid

Handling

	contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. 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. Use Good Laboratory Practices.
Storage:	4 °C

Images



ELISA

Image 1.