IgE GENLISA™ ELISA

REF : KBD1001

Ver 2.0

IVD

Enzyme Immunoassay for the Quantitative Determination of IgE in human serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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CE **REF** KBD1001 Σ 96 tests

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Introduction:

Immunoglobulin E (IgE), is the last of the five classes of human antibodies, produced by plasma cells located in lymph node, has the shortest half-life among them and commonly associated with the various manifestations of allergic disease. IgE plays a vital role in the cross-talk between innate and adaptive immunity. It primarily provides protective immunity against helminth parasites but can also respond to foreign substances even in small amounts and is accepted as a "gate keeper." Diseases which cause the elevation of serum IgE levels include atopic diseases (asthma, allergic rhinitis, atopic dermatitis, urticaria), parasitic diseases, cutaneous diseases, neoplastic diseases, and immune deficiencies. Conditions associated with unusually high serum IgE concentrations (>1,000 IU/mL) are allergic bronchopulmonary aspergillosis, allergic fungal sinusitis, atopic dermatitis, human immunodeficiency virus infection, hyper IgE syndrome, IgE myeloma, lymphoma, systemic parasitosis and tuberculosis

Intended Use:

The IgE GENLISA™ ELISA is intended for the quantitative determination of IgE in human serum and plasma.

Principle:

The IgE GENLISA™ ELISA method employs sandwich enzyme linked immunosorbent assay (ELISA) technique. IgE Monoclonal antibodies are pre-coated onto microwells. Sample and Standards are pipetted into microwells and Human IgE present in the sample are bound by the antibodies. Enzyme conjugate antibody is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution is added to microwells and color develops proportionally to the amount of IgE present in sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated Plate (96 wells) – 1 no
2. Standards (0.5 ml/vial) – 55, 110, 222, 485, 1000 IU/ml
3. Standard 0 (0.0 IU/ml) - 2.0 ml
4. Enzyme Conjugate – 18 ml
5. Control Serum – 0.5 ml
6. (20X) Wash Buffer – 2 x 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10 - 20 minutes; centrifuge for 20 minutes at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10 - 20 minutes; centrifuge for 15 minutes at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. (1X) Wash Buffer Dilution: To make (1X) Wash Buffer, add 25 ml of (20X) Wash Buffer to 475 ml of DI water. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **20 ul of Standards**, **Control Serum**, **Samples** into respective wells in sequence.
3. Add **150 ul of Enzyme Conjugate** to each well. Gently mix.
4. Incubate at 37ºC for 45 minutes in a microplate shaker at approximately 500-800 rpm.
5. Aspirate and wash plate 5 times with (1X) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
6. Add **100 ul** of TMB Substrate into each well.
7. Incubate at room temperature (20-25ºC) in a dark place for 20 minutes
8. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Calculation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown IgE concentrations, find the unknown's Mean Absorbance value 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 IgE Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit, 4-PL or a polynomial curve (2nd order) is best recommended for automated results.

Note:
- If a sample reads more than 1000 IU/ml then dilute it with standard 0. The result obtained should be multiplied by the dilution factor.

Control Serum Range: 120 – 220 IU/ml

Assay Validation
Results of an assay are valid if the following criteria for the controls are met.
The absorbance (OD) of blank value should be not more than 0.100 at 450 nm.
The absorbance (OD) of calibrator 5 should be greater than 1.300.
The absorbance (OD) of control serum should be within established range.

Expected Normal Value
Adult allergy-free population: 0 to 180 IU/ml

Normal value ranges may vary slightly among different laboratories. It is strongly recommended that each laboratory should determine its own range of expected normal values.

Limitations of Method:

Any clinical diagnosis should not be based on the results of in-vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis..

Performance Characteristics:
Sensitivity:
The analytical sensitivity (limit of detection) was calculated by determining the variability of the calibrator 0 based on 12 analyses runs additional 2 x SD. Limit of detection defined at 2.50 IU/ml..

Specificity:
No cross-reactivity to human IgA, IgG, IgM was observed with this assay.

Precision:

	Mean Value IU/ml	SD	%CV
Intra-Assay, sample 1	183.00	7.450	4.10
Inter-Assay, sample 1	178.20	12.180	6.80

Accuracy:
The assay was compared with an enzyme immunoassay as a reference test. The total number of specimens was 266. The values ranged from 0 to 4 469 IU/ml. The least square regression equation and the correlation co-efficient were computed for Genlisa IgE total in comparison with the reference method.

The least square regression analysis was y = 1.015(x) + 14.615 with correlation coefficient of 0.97.

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed

- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

• In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

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EC REP **KinesisDx, Lyoner Strasse 14, Frankfurt, Germany**

Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **20 ul Standards, Control Serum, Samples** into respective wells in sequence.
3	Add **150 ul of Enzyme Conjugate** to each well. Gently mix..
4	Incubate at 37ºC for 45 minutes in a microplate shaker at approximately 500-800rpm.
5	Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
6	Add **100 ul** of **TMB Substrate** into each well.
7	Incubate at room temperature (20-25ºC) in a dark place for 20 minutes
8	Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

SYMBOLS KEY

MTP	Coated Microtiter Plate (12 x 8 wells)
STD	Standard
ENZY CONJ	Enzyme Conjugate
SUB TMB	TMB Substrate
20X WASH BUF	(20X) Wash Buffer
SOLN STOP	Stop Solution
CTRL SERUM	Control Serum
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature