

4. **Linearity**

Three different patient samples were diluted with the "0" calibrator to 1:2, 1:4 and 1:8. IgE values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Serum	Original Value (IU/ml)	Percentage of Recovery		
		1:2	1:4	1:8
1	250	98	102	94
2	125	103	97	96
3	70	101	107	91

REFERENCES

1. Marsh DG, Neely JD, Breazeale DR, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;264:1152-6.
2. Vercelli D. Molecular regulation of the IgE immune response. *Clin Exp Allergy* 1995;25:S2:43-5.
3. Stern A, van Hage-Hamsten M, Sondell K, Johansson SGO. Is allergy screening of blood donors necessary? *Vox Sang* 1995;69:114-9.
4. Hide M, Francis DM, Grattan CEH, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993;328:1599-1604.

2008-01-15

Warning

All of Bio-Quant ELISA Kits have not been tested for clinical use and are not approved in the United States by the FDA for diagnostic clinical use. They are components or reagents made solely for research use, further manufacturing and export use. It is the commitment of Bio-Quant customers to receive its products solely for the purpose of exportation or research, and not for the purposes of clinical diagnostic use.

BIO-QUANT DOES NOT MAKE ANY OTHER WARRANTY OR REPRESENTATION WHATSOEVER, WHETHER EXPRESS OR IMPLIED, WITH RESPECT TO THESE PRODUCTS. IN PARTICULAR Bio-Quant, INC. DOES NOT MAKE ANY WARRANTY OF SUITABILITY, NON-INFRINGEMENT, MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE OF ANY PRODUCT



Immunoglobulin E (IgE) ELISA

Catalog No. BQ 066A (96 tests)

INTENDED USE

The IgE ELISA kit is used for the quantitative measurement of IgE in human serum or plasma.

SUMMARY AND EXPLANATION

IgE constitutes a fraction of the total antibody in serum 50-300 ng/ml (compared to 10 mg/ml for IgG) and together with its Fc receptor is important in primary immune responses. The immunogenetic mechanisms underlying IgE responsiveness seen in the atopic diseases can be divided into antigen-specific and non-antigen-specific responses. IgE antibodies to common antigens are reported in the serum of 13% of normal blood donors. Autoantibodies to the IgE Fc-*epsilon*-RII (high affinity receptors) reported in the sera of patients with chronic urticaria, can induce histamine release from mast cells. Patients with atopic allergic diseases such as atopic asthma, atopic dermatitis, and hay fever have been shown to exhibit increased total immunoglobulin E (IgE) levels in blood. IgE is also known as the reagenic antibody. In general, elevated levels of IgE indicate an increased probability of an IgE-mediated hypersensitivity, responsible for allergic reactions. Parasitic infestations such as hookworm, and certain clinical disorders including aspergillosis, have also been demonstrated to cause high levels of IgE. Decreased levels of IgE are found in cases of hypogammaglobulinemia, autoimmune diseases, ulcerative colitis, hepatitis, cancer, and malaria. Cord blood or serum IgE levels may have prognostic value in assessing the risk of future allergic conditions in children. Certain groups of white blood cells, including basophils and tissue mast cells, have membrane receptors for the IgE molecule.

PRINCIPLE OF THE TEST

The IgE is a two-site sandwich ELISA method. Samples and diluent are added to the wells coated with Anti-IgE MAb. IgE in the patient's serum binds to anti- MAb on the well. Unbound proteins are washed off by wash buffer. Anti-IgE HRP labeled second antibody is then added. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of IgE in the samples. A standard curve is prepared relating color intensity to the concentration of the IgE.

MATERIALS PROVIDED	96 tests
1. Microwell coated with IgE MAb	12x8x1
2. IgE Standard: 6 vials (ready to use)	0.7ml
3. IgE Enzyme Conjugate: 1 bottle (ready to use)	12 ml
4. Assay Diluent: 1 bottle (ready to use)	12 ml
5. TMB Substrate: 1 bottle (ready to use)	12ml
6. Stop Solution: 1 bottle (ready to use)	12ml
7. 20X Wash concentrate: 1 bottle	25ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

- Potential biohazardous materials:
The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
- This test kit is designed for research use only.
- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that serum samples be run in duplicate.
- Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

- Collect blood specimens and separate the serum immediately.
- Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
- Avoid multiple freeze-thaw cycles.
- Prior to assay, frozen sera should be completely thawed and mixed well.
- Do not use grossly lipemic specimens.

REAGENTS PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26° C).

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

- Place the desired number of coated strips into the holder
- Pipet 20 µl of IgE standards, controls and patient's sera.
- Add 100 µl of assay diluent into each well.
- Cover the plate and incubate for 30 minutes at room temperature (18-26° C).
- Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
- Add 100 µl Enzyme Conjugate into each well.
- Cover the plate and incubate for 30 minutes at room temperature (18-26° C).
- Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
- Add 100 µl of TMB substrate to all wells.
- Incubate for 10 minutes at room temperature.
- Add 50 µl of stop solution into each well. Shake the plate gently for 30 seconds to mix the solution. Make sure that the blue color completely changes to yellow.
- Read absorbance on ELISA Reader at 450 nm within 15 minutes from adding stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

- Check IgE standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
- To construct the standard curve, plot the absorbance for the IgE standards (vertical axis) against its concentration in IU/ml (horizontal axis) on a linear graph paper. Draw the best curve through the points.
- Use the absorbance for controls and each unknown sample to determine the corresponding concentration of IgE from the standard curve.

Example of a Standard Curve

	OD 450 nm	Conc. IU/mL
Std 1	0.028	0
Std 2	0.224	25
Std 3	0.612	75
Std 4	1.108	150
Std 5	1.753	300
Std 6	2.477	600

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for IgE may be used as initial guideline ranges only:

IgE Normal Range: Male = Less than 250 IU/ml, Female = Less than 175 IU/ml

LIMITATIONS OF THE TEST

- The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

PERFORMANCE CHARACTERISTICS

- Correlation with a Reference ELISA kit:**

A total of 117 sera were tested by this IgE ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.93	1.0	-9.1

- Precision**

Intra-Assay

Serum	No. of Replicates	Mean IU/ml	Standard Deviation	Coefficient of Variation (%)
1	16	243	120	4.93
2	16	137	8	5.83
3	16	82	6	7.31

Inter-assay

Serum	No. of Replicates	Mean IU/ml	Standard Deviation	Coefficient of Variation (%)
1	10	247	15	6.07
2	10	131	11	8.39
3	10	78	8	10.25

- Sensitivity**

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean IU/ml	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.3	0.2	0.7 IU/ml