

**CALDON BIOTECH INC.  
Immunoglobulin E (IgE)  
ELISA**

Catalog No. IE066A  
(96 tests)

**INTENDED USE**

The CALDON BIOTECH INC (CBI), 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**

- Microwell strips coated with IgE MAb (12x8x1 wells). Total of 96 wells.
- IgE Standard: 6 vials (0.6 mL each). Ready to use.
- Enzyme Conjugate: 1 bottle (12 mL). Ready to use
- Assay Diluent: 1 bottle (12 mL). Ready to use.
- TMB Substrate: 1 bottle (12 mL). Ready to use.
- Stop Solution: 1 bottle (8 mL). Ready to use.
- 10X Wash Concentrate: 1 bottle (50 mL).

**STORAGE AND STABILITY**

- Store the kit at 2 - 8°C.
- Keep microwells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.

**WARNINGS AND PRECAUTIONS**

1. 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
2. This test kit is designed for in vitro diagnostic use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. 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**

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at 2 to 8° C for 5 days. If storage time exceeds 5 days, store frozen at (-20 ° C) for up to one month.

3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

#### REAGENTS PREPARATION

10X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 450 ml of distilled water. Store at room temperature.

#### 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  $\mu$ L of IgE standards, controls and patient's sera.
- Add 100  $\mu$ L 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. Fill wells with working wash buffer. Wash three times. Blot on absorbent paper towels.
- Add 100  $\mu$ L Enzyme Conjugate into each well.
- Cover the plate and incubate for 30 minutes at room temperature (18-26° C).
- Remove liquid from all wells. Fill wells with working wash buffer. Wash three times. Blot on absorbent paper towels.
- Add 100  $\mu$ L of TMB substrate to all wells.
- Incubate for 10 minutes at room temperature.
- Add 50  $\mu$ L 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 30 minutes from adding stop solution.

#### CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. 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.
2. To construct the standard curve, plot the absorbance for the IgE standards (vertical axis) against its concentration in ng/mL (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Use the absorbance for controls and each unknown sample to determine the corresponding concentration of IgE from the standard curve.

#### 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

1. 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.

2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

#### PERFORMANCE CHARACTERISTICS

##### 1. 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

##### 2. Precision Intra-Assay

Serum	No. of Replicates	Mean IU/mL	Standard Deviation	Coefficient of Variation%
High	16	243	120	4.93
Normal	16	137	8	5.83
Low	16	82	6	7.31

##### Inter-assay

Serum	No. of Replicates	Mean IU/mL	Standard Deviation	Coefficient of Variation%
High	10	247	15	6.07
Normal	10	131	11	8.39
Low	10	78	8	10.25

##### 3. 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

#### 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.