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Cat#: IB19111 (96 Tests)
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Immunoglobulin E (IgE) ELISA

RUO

Catalog No. IB19111 (96 Tests)

INTENDED USE

The IgE ELISA Kit is intended for the determination of IgE in human serum.
For research use only, not for use in diagnostic procedures.

PRINCIPLE OF THE TEST

The IgE is a solid phase sandwich assay method, based on a streptavidin-biotin principle. The standards, samples and biotinylated anti-IgE antibody reagent are added into designated wells coated with Streptavidin. Endogenous IgE in the serum binds to the antigenic site of the biotinylated anti-IgE antibody. Simultaneously, the biotinylated antibody is immobilized onto the wells through the high affinity streptavidin-biotin interaction. Unbound protein and excess biotin conjugated antibody are washed off by wash buffer. Upon the addition of the HRP conjugated anti-IgE antibody reagent, a sandwich complex is formed, the analyte of the interest being in between the two highly specific antibodies, labelled with biotin and HRP. Unbound excess enzyme conjugated antibody reagent is washed off by wash buffer. Upon addition of the substrate, the intensity of color developed is directly proportional to the concentration of the 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 Streptavidin	12x8x1
2. IgE Standard: 6 vials (ready to use)	0.5ml
3. IgE Biotin Conjugate: 1 bottle (ready to use)	12 ml
4. IgE Enzyme Conjugate: 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

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. 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
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that serum samples be run in duplicate.
7. 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. Typically, 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.
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.

REAGENT 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 (20-25°C).

ASSAY PROCEDURE

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

1. Place the desired number of coated strips into the holder
2. Pipette 25uL of IgE standards, controls, and samples in to appropriate wells.
3. Add 100uL of Biotin Reagent into each well. Shake the plate for (10-30) sec.
4. Cover the plate and incubate for 30 minutes at room temperature (20-25°C).
5. Remove liquid from all wells. Wash wells three times with 300uL of 1X wash buffer. Blot on absorbance paper or paper towel.
6. Add 100uL of Enzyme Reagent into each well.
7. Cover the plate and incubate for 30 minutes at room temperature (20-25°C).
8. Remove liquid from all wells. Wash wells three times with 300uL of 1X wash buffer. Blot on absorbance paper or paper towel.
9. Add 100uL of TMB substrate to all wells.
10. Incubate for 15 minutes at room temperature.
11. Add 50uL of stop solution to all wells. Shake the plate gently to mix the solution.
12. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

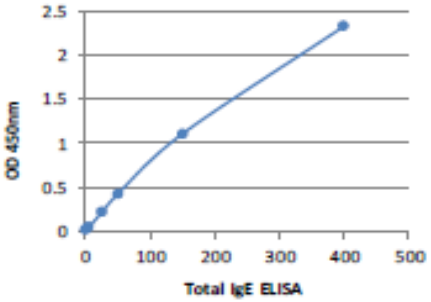
CALCULATION OF RESULTS

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 IU/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.

Example of a Standard Curve

Standard, IU/ml	OD 450NM
0	0.014
5	0.05
25	0.22
50	0.43
150	1.11
400	2.33



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 activit