



# **Human Immunoglobulin E (IgE) OneStep ELISA Kit**

**Catalog number: EK7093**

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

## Human Immunoglobulin E (IgE) OneStep ELISA Kit

**Catalog Number:** EK7093

**Size:** 96 wells/kit

**Sample Type:** Serum and Plasma

**Sensitivity:** 40 IU/ml

**Assay Range:** 40-640 IU/ml

**Storage:** Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

### Introduction

The Bosterbio OneStep ELISA kit is a solid phase sandwich ELISA kit. Instead of incubating with standards, samples and controls and with enzyme conjugated detection antibody separately, the OneStep ELISA kit allows the user to add standards, samples and controls to wells without incubation, and an enzyme conjugate reagent is added into each well immediately. Then incubate standards, samples, controls and enzyme conjugate reagent together. After the excess enzyme conjugate is washed out, the substrate is added into each well. The enzyme catalyzes the substrate yielding a blue color ( $A_{max} = 370\text{nm}$  and  $652\text{nm}$ ) that changes to yellow ( $A_{max} = 450\text{nm}$ ) upon addition of a sulfuric or phosphoric acid stop solution. The intensity of color developed is directly proportional to the concentration of target protein in the samples. A standard curve is generated relating color intensity to the concentration of target protein.

### Kit Components

Description	Quantity
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 Required, but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. ELISA reader capable of reading absorbance at 450nm

5. Absorbance paper or paper towel

6. Graph paper

## WARNINGS AND PRECAUTIONS

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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 AND HANDLING

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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

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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

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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. 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. 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

(See Reagent Preparation Section). Blot On Absorbent Paper Towels.

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 OD for each C-Peptide standard point (vertical axis) versus 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 CA15-3 in U/ml from the standard curve.
- 00.511.522.50100200300400500OD 450nmTotal IgE ELISA

## LIMITATION 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.

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