# Human total IgE ELISA Kit

Enzyme Immunoassay for the quantification of total Immunoglobulin-E (IgE) in serum and plasma

Catalog number: ARG80774

Package: 96 wells

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**MANUFACTURED BY:**

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## INTRODUCTION

The existence of IgE in man as a unique class of immunoglobulins which are important in the mediation of the allergic response has been known for over twenty years. The mechanism of action involves an initial antigenic stimulation of immunocompetent B lymphocytes by a specific antigen, a process which induces the lymphocyte to respond by producing specific antibody of several classes.

One class, reaginic or IgE antibody, becomes partially bound via its Fc portion to receptors on the surface of mast cells and basophilic leukocytes. Upon further stimulation by specific allergens, these cell-bound IgE molecules bind via their Fab portion to the allergen. This combination triggers the mast cells and basophilic leucocytes to release various vasoactive amines into the blood and the surrounding tissue. These substances cause smooth muscle constriction and lead ultimately to allergic conditions such as wheal and flare reactions, hives, dermatitis, rhinitis, hay fever, asthma and anaphylactic shock. IgE determinations are most valuable in the diagnostic assessment of patients with established or suspected allergic desease. In normal subjects, IgE values are related to age, with normal values peaking around 10- 14 years. Infants and children with family history of atopic allergy are at increased risk of developing disease and constitute a prime population for screening. Studies have shown that conditions such as asthma, rhinitis, eczema, urticaria, dermatitis and some parasitic infections lead to increased IgE levels. Asthma, hay fever and atopic eczema patients may produce levels 3- 10 times those of normal patients.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A monoclonal mouse anti-human IgE antibody has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells together with HRP-conjugated anti-human IgE. After washing away any unbound substances, a substrate solution (TMB) is then added to the wells and color develops in proportion to the amount of IgE bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of IgE in the sample is then determined by comparing the O.D of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| antibody-coated microplate | 12 X 8 strips | 4°C |
| Standard 0 (0 IU/ml) | 1 X 1 ml (Ready-to-use) | 4°C |
| Standard 1-5<br>(5, 25, 100, 250, 1000 IU/ml) | 5 X 200 µl (Ready-to-use) | 4°C |
| HRP-Conjugated anti-IgE | 22 ml (Ready-to-use) | 4°C |
| 10X Wash buffer | 60 ml | 4°C |
| TMB substrate | 12 ml (Ready-to-use) | 4°C (Protect from light) |
| STOP solution | 12 ml (Ready-to-use) | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times and do not use after the expiry date.
- After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 4°C.
- Return the unused microtiter strips to the plastic bag and store them dry at 4-8°C
- If crystals are observed in the 10X Wash buffer, warm up to 37°C for 15 min or until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately. The samples can be stored at 2-8 °C up to 2 days or aliquot and store samples at ≤ -20 °C for longer storage. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately. The samples can be stored at 2-8 °C up to 2 days or aliquot and store samples at ≤ -20 °C for longer storage. Avoid repeated freeze-thaw cycles.

Note:

- Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.
- For the performance of the test the samples and the standards have to be used undiluted.

## REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 60 ml of 10X wash buffer + 540 ml of distilled water) The diluted wash buffer can be kept for 4 weeks at 2-8°C.

## ASSAY PROCEDURE

All reagents and samples must be brought to room temperature (18-25°C) before use, but should not be left at this temperature longer than necessary. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. And store them dry at 4-8°C
2. Add **10 µl** of each standard (ready-to-use) and sample (undiluted) in duplicate into wells.
3. Add **200 µl** of **HRP-Conjugated anti-IgE** to each well. Leave one well empty for the substrate blank.
4. Cover wells and **incubate for 30 mins at RT**.
5. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µl** of **TMB Substrate Reagent** to each well. This time also the substrate blank well is pipetted. Incubate for **15 minutes at room temperature** in dark.
7. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
8. **Read the OD** with a microplate reader at **450 nm** immediately. (Optional: read at 620 nm as reference wavelength). The color is stable for at least 60 minutes.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. Any sample reading greater than the highest standard should be diluted appropriately with zero standard and re-assayed. The result has to be multiplied by the dilution factor.

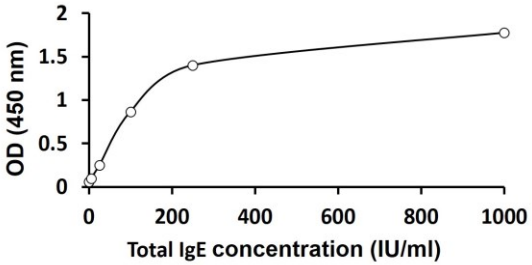
7. Result Interpretation:

Normal ranges of Total IgE are dependent on age:

| Age | Normal Range [IU/mL] |
|---|---|
| Newborns | < 1.2 |
| 1 – 6 months | < 7.2 |
| 7 – 12 months | < 12.7 |
| 1 – 5 years | < 60 |
| 6 – 9 years | < 155 |
| 10 – 15 years | < 199 |
| Adults (Greyzone) | < 100 (60-100) |

## EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.




Acceptable range:

| Standard | Conc. IU/ml | Acceptable OD range: |
|---|---|---|
| Standard 0 | 0 | ≤ 0.100 |
| Standard 1 | 5 | ≥ 0.015 |
| Standard 2 | 25 | ≥ 0.050 |
| Standard 3 | 100 | ≥ 0.150 |
| Standard 4 | 250 | ≥ 0.400 |
| Standard 5 | 1000 | ≥ 1.000 |

## QUALITY ASSURANCE

### Sensitivity

The minimum detectable dose (MDD) of total IgE ranged from 5-1000 IU/ml. The mean MDD was 0.8 IU/ml.

### Cross-Reactivity

No cross-reactivity to Immunoglobulin G.

### Specificity and Sensitivity

100%

### Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5.1% and inter-assay precision was 4.1%.

### Inter-lot Precision

1.3-5.9%

### Recovery

87-97%

### Linearity

95-126%å

### Interferences

No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL