# ab178659 – Immunoglobulin E (IgE) Human ELISA Kit

Instructions for Use

An immunoenzymatic assay for the quantitative measurement of Immunoglobulin E (IgE) in Human serum and plasma.

This product is for research use only and is not intended for diagnostic use.

Version 4b Last Updated 8 August 2024

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## 1. BACKGROUND

Abcam's Immunoglobulin E (IgE) Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of IgE in serum and plasma.

The essential reagents required for an immunoenzymometric assay include high affinity and specific antibodies. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-IgE antibody.

Upon the mixing the monoclonal biotinylated antibody and a serum containing the native antigen, the native antigen and the antibody forms an antibody-antigen complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration.

Another antibody (directed at a different epitope) labelled with an enzyme (HRP) is added. Another interaction occurs forming an enzyme labelled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off. A suitable substrate is added to produce colour, which is measured using a microplate spectrophotometer. The enzyme activity in the well is directly proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be determined.

IgE is an antibody isotype, found only in mammals. Although IgE is typically the least abundant isotype - blood serum IgE levels in a normal ("non-atopic") individual are ~150ng/ml, compared to 10mg/ml for the IgGs (the isotypes responsible for most of the classical adaptive immune response) - it is capable of triggering the most powerful immune reactions. Most of our knowledge of IgE has come from an allergy known as type 1 hypersensitivity.

IgE plays an important role in allergy, and in the immune system's recognition of cancer.

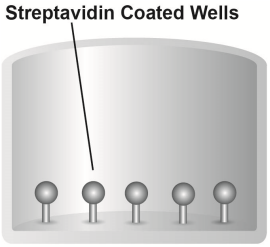
People who suffer from true IgE-mediated allergies can have up to 10 times the normal level of IgE in their blood (as do sufferers of hyper-IgE syndrome).

The IgE molecules (MW 200,000) bind to the surface of the mast cells and basophilic granulocytes. Subsequently the binding of allergen to cell-bound IgE causes these cells to release histamine and other vasoactive substances. The release of histamines in the body initiates what is commonly known as an allergic reaction.

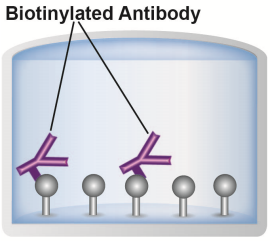
IgE levels show a slow increase during childhood, reaching adult levels in the second decade of life. In general, the total IgE levels increase with the allergies a person has and the number of times of exposure to the relevant allergens. Significant elevations may be seen in the sensitized individuals, but also in cases of myeloma, pulmonary aspergillosis, and during the active stages of parasitic infections.

The measurement of immunoglobulin E (IgE) in serum is widely used in the diagnosis of allergic reactions and parasitic infections. Before making any therapeutic determination, it is important to know whether the allergic reaction is IgE mediated or non-IgE mediated. Measurement of total IgE in serum sample, along with other supporting diagnostic information, can help to make that determination.

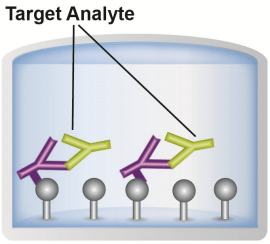
## 2. ASSAY SUMMARY



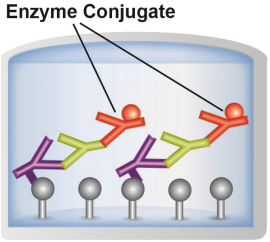

Prepare all reagents, samples and standards as instructed.



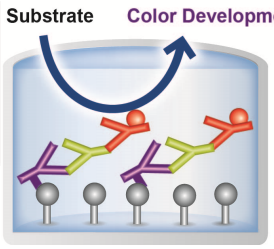

Add Biotinylated Antibody to appropriate wells.




Add standard or sample to appropriate wells. Incubate at room temperature.




After washing, add Enzyme Conjugate to appropriate wells. Incubate at room temperature.




After washing, add TMB Substrate Solution to each well. Incubate at room temperature. Add Stop Solution. Read at 450 nm.

## 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

## 4. STORAGE AND STABILITY

**Store kit at 2-8ºC immediately upon receipt.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

## 5. MATERIALS SUPPLIED

| Item | Amount | Storage Condition (Before Preparation) |
|---|---|---|
| Streptavidin Coated Microplate (12 x 8 wells) | 96 Wells | 2-8ºC |
| Stop Solution | 15 mL | 2-8ºC |
| IgE Biotin Conjugate | 13 mL | 2-8ºC |
| TMB Substrate Solution | 15 mL | 2-8ºC |
| 50X Washing Solution | 20 mL | 2-8ºC |
| IgE HRP Conjugate | 13mL | 2-8ºC |
| IgE Standard 0 – 0 IU/mL | 1 mL | 2-8ºC |
| IgE Standard 1 – 5 IU /mL | 1 mL | 2-8ºC |
| IgE Standard 2 – 25 IU /mL | 1 mL | 2-8ºC |
| IgE Standard 3 – 50 IU /mL | 1 mL | 2-8ºC |
| IgE Standard 4–150 IU /mL | 1 mL | 2-8ºC |
| IgE Standard 5– 400 IU /mL | 1 mL | 2-8ºC |
| Strip Holder | 1 unit | 2-8ºC |
| Cover Foil | 1 unit | 2-8ºC |

## 6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer

## 7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

## 8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

## 9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 **1X Washing Solution**

Prepare 1X Washing Solution by diluting 50X Washing Solution with deionized water. To make 500 mL 1X Washing Solution combine 10 mL 50X Washing Solution with 490 mL deionized water. Mix thoroughly and gently.

- All other solutions are supplied ready to use

## 10. SAMPLE COLLECTION AND STORAGE

- Use Human serum or plasma samples with this assay. If the assay is performed within 5 days of sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing.
- Samples with concentration over 400 IU/mL should be diluted with Standard 0.

*Avoid repeated freezing and thawing.*

## 11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

## 12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**

12.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

12.3. Add 25 µL standards, control and samples into their respective wells. Add 100 µL IgE biotin conjugate to each well. Leave a blank well for substrate blank.

12.4. Cover wells with the foil supplied in the kit and incubate for 30 minutes at room temperature.

12.5. Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining 1X Washing Solution by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid

Note: Complete removal of liquid at each step is essential for good assay performance.

12.6. Add 100 µL IgE HRP conjugate into all wells except the blank.

12.7. Cover wells with the foil supplied in the kit and incubate for 30 minutes at room temperature.

12.8. Repeat step 12.5.

12.9. Add 100 µL TMB Substrate Solution into all wells.

12.10. Incubate for exactly 15 minutes at room temperature in the dark.

12.11. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.

12.12. Measure the absorbance of the sample at 450 nm within 5 minutes of addition of the Stop Solution.

*Dual wavelength reading using 620 nm as reference wavelength is recommended.*

## 13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e.g. Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations.

**OD COVERSION -** The optical densities (O.D.s) of some calibrators and samples may be higher than 2.0, in such a case, they could be out of the measurement range of the microplate reader. It is therefore necessary, for O.D.s higher than 2.0, to perform a reading at 405 nm (=wavelength of peak shoulder) in addition to 450 nm (peak wavelength) and 620 (reference filter for the subtraction of interferences due to the plastic).

For microplate readers unable to read the plate at 3 wavelengths at the same time, it is advisable to proceed as follows:

- Read the microplate at 450 nm and at 620 nm.

- Read again the plate at 405 nm and 620 nm.

- Find out the wells whose ODs at 450 nm are higher than 2.0

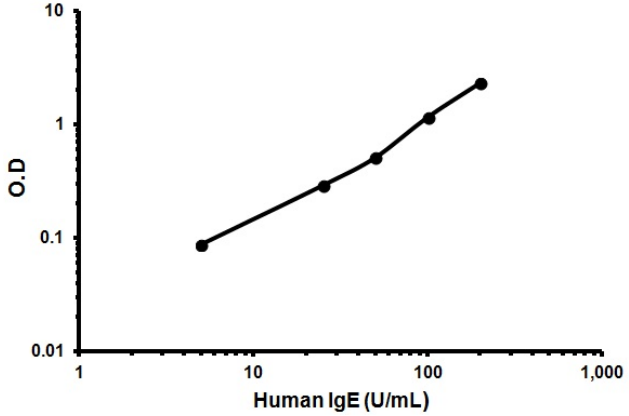
- Select the corresponding ODs read at 405 nm and multiply these values at 405 nm by the conversion factor 3.0 (where OD 450/OD 405 = 3.0), that is: OD 450 nm = OD 405 nm x 3.0.

Warning: The conversion factor 3.0 is suggested only. For better accuracy, the user is advised to calculate the conversion factor specific for his/her own reader.

**VALIDATION** – Maximum absorbance of standard 5 should be ≥ 1.0.

## 14. TYPICAL DATA

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



## 15. TYPICAL SAMPLE VALUES

**REFERENCE VALUES –**

The serum IgE values are shown in the table below:

| Age | Median | Range (IU/mL) |
|---|---|---|
| 0-3 | 6.4 | 0 – 46 |
| 3-16 | 25.0 | 0 – 280 |
| Adults | 43.0 | 0 - 200 |

**PRECISION –**

| | Intra-Assay | Inter-Assay |
|---|---|---|
| n= | 16 | 16 |
| %CV | ≤ 7.2 | ≤ 7.6 |

**ACCURACY –**

The recovery has been performed by adding 50 – 100 – 200 IU/ml of IgE to three samples. The results are reported in the table below:

| Sample | Measured IU/mL | Recovered IU/mL | % Recovery |
|---|---|---|---|
| Pool 1 | 10.6 | | |
| Pool1 + 50 | 61.3 | 50.7 | 101.2 |
| Pool1 + 100 | 116.2 | 105.6 | 105.6 |
| Pool1 + 200 | 209.1 | 198.5 | 99.3 |
| Pool2 | 65.8 | | |
| Pool2 + 50 | 112.3 | 46.5 | 93.0 |
| Pool2 + 100 | 165.6 | 99.8 | 99.8 |
| Pool2 + 200 | 258.1 | 192.3 | 96.2 |
| Pool3 | 25.3 | | |
| Pool3+ 50 | 76.3 | 51.0 | 102.0 |
| Pool3 +100 | 122.5 | 97.2 | 97.2 |
| Pool3 + 200 | 225.2 | 199.9 | 100.0 |

**SENSITIVITY –**

The minimal detectable concentration of IgE by this assay is estimated to be 0.27 IU/ml at the 95 % confidence limit.

**CORRELATION –**

Abcam's Immunoglobulin E (IgE) Human ELISA Kit was compared to a commercially available IgE kit.

214 serum samples were tested

The regression curve is:

$y = 1.175 x - 11.172$

$r^2 = 0.972$

y = IgE Competitor kit

x = IgE Abcam's kit

## 16. ASSAY SPECIFICITY

In order to assess the specificity of the antibody pair used for the IgE Elisa assay, massive doses of related analytes were spiked in a pool of patient sera:

| Cross Reagent | U.M. | Tested Concentration | Cross Reactivity |
|---|---|---|---|
| IgE | IU/mL | | 100 % |
| IgA | IU/mL | 1000 | None detected |
| IgM | IU/mL | 1000 | None detected |
| IgG | IU/mL | 1000 | None detected |

## 17. TROUBLESHOOTING

| Problem | Cause | Solution |
|---|---|---|
| Low signal | Incubation time to short | Try overnight incubation at 4 °C |
| | Precipitate can form in wells upon substrate addition when concentration of target is too high | Increase dilution factor of sample |
| | Using incompatible sample type (e.g. serum vs. cell extract) | Detection may be reduced or absent in untested sample types |
| | Sample prepared incorrectly | Ensure proper sample preparation/dilution |
| Large CV | Bubbles in wells | Ensure no bubbles present prior to reading plate |
| | All wells not washed equally/thoroughly | Check that all ports of plate washer are unobstructed/wash wells as recommended |
| | Incomplete reagent mixing | Ensure all reagents/master mixes are mixed thoroughly |
| | Inconsistent pipetting | Use calibrated pipettes & ensure accurate pipetting |
| | Inconsistent sample preparation or storage | Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles) |

| Problem | Cause | Solution |
|---|---|---|
| High background | Wells are insufficiently washed | Wash wells as per protocol recommendations |
| | Contaminated wash buffer | Make fresh wash buffer |
| | Waiting too long to read plate after adding stop solution | Read plate immediately after adding stop solution |
| Low sensitivity | Improper storage of ELISA kit | Store all reagents as recommended. Please note all reagents may not have identical storage requirements. |
| | Using incompatible sample type (e.g. Serum vs. cell extract) | Detection may be reduced or absent in untested sample types |

## 18. NOTES

## Technical Support



For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)