Total IgE ELISA Kit

Quantitative Assay for Total IgE Antibodies

Product code GD09

96 tests

Reagents included in the kit are for *in vitro* diagnostic use only

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1. Intended use

The total IgE kit is a sensitive ELISA method for the detection of IgE class antibodies. It is intended as an aid to diagnosis of atopy in children and adults. The components of the kit are for *in vitro* diagnostic use only.

2. Introduction.

Immunoglobulin E has a molecular structure similar to that of other classes, having two specific antigen binding sites attached to a constant (Fc) region. IgE in serum has no known biological relevance and exerts its effect only when bound to blood basophils or tissue mast cells. Mediators are released from these cells are responsible for the immediate hypersensitivity reactions.

The mechanisms regulating IgE synthesis differs from those of the other classes. Serum levels are generally low in normal individuals. Levels rise in childhood to reach adult levels by the age of 15 to 20 years. Because IgE levels are usually low, a specific IgE response to an allergen will often cause a significant rise in total IgE.

The assay is calibrated against the Second International IgE Reference Serum: 75/502 (IU/ml). An elevated total IgE level is strongly suggestive of an atopic predisposition, but gives no clue to the causative agent. Raised levels are found in patients with parasitic disease, tropical eosinophilia and during certain drug reactions.

The measurement of total IgE is most helpful in diagnosis of atopic disease in young children. In adults there is an overlap between atopic and normal subjects. Values up to 120 IU/ml are found in the normal population and most patients with atopic disease have levels above 120 IU/ml. There is no gender related difference in normal IgE concentrations.

3. Principle of the test

Diluted serum samples are incubated with monoclonal anti human IgE immobilised on microtitre wells. After washing away unbound serum components, monoclonal mouse anti-human IgE conjugated to horseradish peroxidase is added to the wells, and this binds to surface-bound IgE during the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of stop solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the standards, positive control and samples are measured using a microplate reader at 450nm.

4. Materials included in the kit

- **Microplate:** 96 wells in 12 X 8 break-apart strips, pre-coated with monoclonal anti-IgE, with holder in a foil bag with desiccant
- **Reagent 1**: **Sample Diluent** 10mM Buffered protein solution, pH 7.2 with antimicrobial agent, 50 ml, (blue), ready to use
- **Reagent 2: Wash Buffer Concentrate** (X 10), 100mM Tris-buffered saline with detergent, pH 7.2, 100ml
- **Reagent 3: Conjugate** mouse anti-human IgE conjugate (horseradish peroxidase) in protein stabilising solution and antimicrobial agent, 12 ml, ready to use
- **Reagent 4: TMB Substrate** aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- **Reagent 5: Stop Solution** 0.25M sulphuric acid, 12 ml, ready to use
- **Standards:** 0**,** 50, 150, 375, 1250 IU/ml, 1ml of 10mM Tris-buffered saline containing human serum IgE, ready to use
- **Positive Control:** 1ml of 10mM Tris-buffered saline containing human serum IgE, ready to use
- **Instructions for use**

5. Other equipment required

1. Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 10µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable automated system may be used.
2. Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

6. Precautions

6.1 Safety Precautions

1. All reagents in this kit are for *in vitro* diagnostic use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. All human source material used in the preparation of standards and control for this product have been tested and found negative for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local legislation.

6.2 Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 – 8^{o}C after use.
3. Include the positive control in every test run to monitor for reagent stability and correct assay performance.
4. Strictly observe the indicated incubation times and temperature.
5. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
6. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for enzyme conjugate completely separate from the substrate reagent.
7. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
8. Do not allow microwells to dry between incubation steps.
9. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
10. Avoid direct sunlight and exposure to heat sources during all incubation steps.
11. Replace colour-coded caps on their correct vials to avoid cross-contamination
12. It is important to dispense all samples and the positive control into the wells without delay. Therefore ensure that all samples are ready to dispense.

7. Shelf life and storage conditions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for three months (or until its expiry date if less than three months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer has a shelf life of 3 months if stored in a closed bottle at 2 – 8°C.

8. Specimen collection and storage

Serum or plasma samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

9. Preparation of reagents

1. Dilute the Wash Buffer (**Reagent 2)** 1: 9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water.

10. Assay Procedure

Assemble the number of strips required for the assay.

1. Dispense 100 µl of each standard and positive control. Dispense 20µl of each sample to be tested. Dispense 80µl of sample diluent into each sample well (to make a 1/5 dilution). Tap the plate rapidly to mix the well contents.

2. Incubate for 6**0** minutes at room temperature. During all incubations, avoid direct sunlight and close proximity of any heat sources.

3. After 60 minutes, decant or aspirate the well contents and wash the wells 3 times using automated washing or the manual wash procedure (see below). Careful washing is the key to good results. **Do not allow the wells to dry out.**

   Manual Wash Procedure:
   Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.

4. Dispense 100µl of Conjugate (**Reagent 3)** into each well. Incubate the wells for **30** minutes at room temperature.

5. After 30 minutes, discard the well contents and carefully wash the wells 4 times with wash buffer. Ensure that the wells are empty but do not allow to dry out.

6. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate (**Reagent 4)** into each well. Incubate the plate for **10** minutes.

7. Add 100µl of Stop Solution (**Reagent 5)** to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.

8. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

11. Quality control

Quality control data is supplied on the lot-specific QC certificate included in the kit.

The positive control is intended to monitor for substantial reagent failure.

Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD-values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

12. Interpretation of Results

Using a 4 parameter logistic fit; plot the optical density of each standard against its concentration. Read the unknowns off this curve. Values above 1250 IU/ml should be repeated at a higher dilution, using the sample diluent provided.

Reference Ranges
The following normal ranges (mean + 2 Standard deviations) are given for guidance only as laboratories should always establish their own reference range. Geographical factors may influence the results.

Age	Values (IU/ml 75/502)
Newborn	< 11
Up to 3 months	< 25
3 to 12 months	< 37
1 to 5 years	< 135
5 to10 years	< 144
Adult	< 188

Patients with IgE values below the above age related levels have a low probability of atopic disease. Results must be interpreted in conjunction with other clinical information relating to each patient.
Assay sensitivity
The minimum detectable concentration of Total IgE is 0.9 IU/ml

13. Limitations of the Procedure

1. Results of this assay should be interpreted in conjunction with clinical findings and patient history.
2. The IgE response is short lived. Patients who have not had a recent atopic challenge may show low IgE levels.
3. Results of this assay are not diagnostic proof of the presence or absence of atopic disease.

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14. Performance Characteristics

Inter-assay precision

	IgE IU/ml	CV%
Sample A	497	3.0
Sample B	614	3.1
Sample C	2013	4.7

Intra-assay precision
CVs typically <12%

Linearity
Serial dilutions of a serum yielded a linear response.

Dilution	Measured IgE	Corrected IgE
Neat	784	784
1:1	387	774
1:2	191	764
1:4	98	784

Method Summary

- Dispense 100µl standards and the Positive Control
- **Dispense 20**µl **of each test sample.**
- Dispense 80µl of sample diluent into each sample well **(Reagent 1).**
- Incubate for 6**0** minutes at room temperature.
- *Wash the wells three times*
- Dispense 100µl of Conjugate **(Reagent 3)** into each well
- Incubate at room temperature for **30** minutes
- *Wash the wells four times*
- Add 100µl of TMB Substrate **(Reagent 4)** to each well
- Incubate at room temperature for **10** minutes
- Add 100µl Stop Solution **(Reagent 5)** to each well
- Read the optical density at 450nm (single wavelength) or 450/620nm (dual wavelength).

Further reading

Pollart SM, Smith TF, Morris EC, et al. Environmental exposure to cockroach allergens: analysis with monoclonal antibody-based enzyme immunoassays. J Allergy Clin Immunol 1991;87:50510.
Chapman MD. Allergen specific monoclonal antibodies: new tools for the management of allergic disease. Allergy 1988;43:714.
Kemeny DM, Urbanek R, Samuel D, et al. Increased sensitivity and specificity of a sandwich **ELISA** for measurement of **IgE** antibodies. J Immunol Methods 1985;78:21726.
Kemeny DM, Urbanek R, Samuel D,et al. The use of monoclonal and polyspecific antibodies in the **IgE** Sandwich **ELISA**. J Immunol Methods 1986;87:4550.