Version 20-04/24

User's Manual

Food Allergen specific IgE ELISA Kit

REF **DEIAGEF-01**

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RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Allergen specific IgE ELISA Kit is for in vitro qualititative measurement of IgE in human serum. The food allergen includes milk, peanuts, egg, soy bean, wheat, tomato, fish, pawn, crab and nuts.

General Description

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.

Principles of Testing

This kit employs solid phase, ELISA assay for detection of IgG antibodies to food allergen in three-step incubation procedure. Polystyrene microwell strips are pre-coated with purified food allergen antigens. During the first incubation step, food allergen IgG specific antibodies, if present, will be bound to the solid phase precoated antigen complexes. The wells are washed to remove unbound serum proteins, and anti-IgG antibodies (anti-IgG) conjugated to biotin is added. During the second incubation step, these biotin- labelled antibodies will be bound to any antigen-IgG complexes previously formed and the unbound -conjugate is then removed by washing. After washing, Streptavidin-HPR is added to bound with biotin. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for IgG antibodies to food allergen remain colorless.

Reagents And Materials Provided

1. ELISA microplate 1pc
2. Anti-Human IgE Antibody-Biotin 2 × 5mL
3. Streptavidin-HRP 2 × 5mL
4. Concentrated Wash Buffer 2 × 25mL
5. Stop Solution 1 × 6mL
6. Substrate A 1 × 6mL
7. Substrate B 1 × 6mL
8. Sample Buffer 1 × 6mL
9. IgE Positive Control 1 × 1.3mL
10. IgE Negative Control 1 × 1.3mL

Materials Required But Not Supplied

1. 100 - 1000 µL micropipets
2. Volumetric flask
3. Analytical balance
4. Mortar, mixer
5. Water bath
6. Centrifuge
7. ELISA reader

Storage

Stored at 2-8°C, 6 months

Specimen Collection And Preparation

This kit can be used for detection of serum samples.

Blood was drawn and transferred to vacuum tubes. Solidify at room temperature for more than 30 minutes (it is best not to add anticoagulant, and place it at 2-8°C to solidify naturally). When the clot begins to shrink, immediately separate the serum from the whole blood sample (recommended to use a high-speed refrigerated centrifuge at 3000Xg for 10 min), and then transfer the serum to an appropriate storage tube.

Serum samples can be stored at 2-8°C for up to 7 days. For prolonged storage please keep at -20°C.

Severely hemolyzed or lipemic sera should be avoided.

All samples must be equilibrated to room temperature (22-28°C) and mixed by gentle inversion or vortexing before using samples for assay.

Serum samples that are microbially contaminated, heat-treated, and contain obvious particulates should not be used for testing.

Assay Procedure

1. Prepare all the reagent in the room temperature.
2. Pipet 100µL IgE Negative Control to negative control well; Pipet 100µL IgE Positive Control to positive control well. Add 50µL Sample Buffer to testing well first, and add 50µL sample afterwards.
3. Shake the microplate gently. Seal the microplate with self-adhesive paper, and incubate the sealed microplate in 37 °C water bath for 1 hour
4. Empty the wells. Wash 3- times by filling each well with 200~300µL diluted washing buffer.
5. Add 100µL Anti-Human IgE Antibody-Biotin the testing well. Incubate the microplate at 37 °C for 1 hour. Repeat Step 4.
6. Add 100µL Streptavidin-HRP the testing well. Incubate the microplate at 37 °C for 0.5 hour. Repeat Step 4.
7. Add 50µL Substrate A and Substrate B to wells. Incubate the microplate at 37 °C for 10 mins. Pipet 50µL Stop Solution afterwards.
8. Measure absorbance in each well using a spectrophotometric microplate reader at dual wavelengths of 450/630 nm.

Note: Please finish reading OD in 20mins after adding Stop Solution

Quality Control

Test Negative Control: $OD_{Neg.}$ < 0.1

Test Positive Control: $OD_{Pos.}$ > 0.1

Conformity rate of negative reference product

Except for the negative quality control and positive quality control detection wells, test the corresponding 10 negative reference samples for other detection wells, and the results should all be negative.

Conformity rate of positive reference product

Except for negative quality control and positive quality control detection wells, test the corresponding 10 positive reference samples for other detection wells, and the results should all be positive.

Interpretation Of Results

OD<0.15, Negative

OD≥0.15 Positive

1. A definitive results of food intolerance should not be based only on the results of a singlediagnostic method. In vitro evidence of IgE should always be accompanied by full medical history and analysis of symptoms.
2. Each person reacts individually, thus identical results in the test do not automatically imply the same diagnosis. Different foods with similar molecular structures or epitopes may trigger weak or severe cross-reactions. Cross-reactions must always be considered.
3. Negative in vitro results may occasionally occur in person with food intolerance symptoms that clearly correlate with food contact.
4. Sensitization to foodstuffs not tested in the ELISA cannot be excluded.
5. The binding capacity for IgE antibodies may vary from food to food. Therefore, identical results for different foods may not necessarily apply exactly equal IgE antibody levels.

Precision

Intra-assay CV: ≤15%

Inter-assay CV: ≤15%

Sensitivity

The minimum detection limit of allergen-specific IgE antibodies is 0.35 IU/. The minimum detection limit for total IgE antibodies was 3 IU/mL.

The HOOK effect will not occur when the allergen-specific IgE concentration is lower than 80.0 IU/mL, and the HOOK effect will not occur when the total IgE concentration is lower than 600 IU/mL

Specificity

Cross-reaction substances:

Baichenduan: dust, shrimp, crab, cockroach, snail

Dwarf ragweed: mugwort, banana, giant porpoise

Alternaria: aspergillus fumigatus, Mycospora

Cat dander: dog dander, horse dander, cow dander

Precautions

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
5. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
6. Storing the concentrated Wash Buffer at 2 - 8°C might lead to formation of salt crystals. If so, resolve those salt crystals by gentle warming (max 40°C) of the buffer solution prior the dilution.
7. Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
8. Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
9. During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

   In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
10. Personal precautions, protective equipment and emergency procedures:

    Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

    Exposure controls/personal protection: Wear protective gloves of nitril rubber or natural latex.

    Wear protective glasses. Used according to intended use no dangerous reactions known.