Version 10-09/22

User's Manual

Inhalant Allergen specific IgE ELISA Kit

REF **DEIAGEI-01**

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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.

Creative Diagnostics

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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 inhalant allergen includes Dermatophagoides pteronyssinus, house dust, cockroach, cat epithelium, dog epithelium, moulds, tree pollens, white mulberry and short ragweed.

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 pre-coated 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 x 5mL
3. Streptavidin-HRP 2 x 5mL
4. Concentrated Wash Buffer 2 x 25mL
5. Stop Solution 1 x 6mL
6. Substrate A 1 x 6mL
7. Substrate B 1 x 6mL
8. Sample Buffer 1 x 6mL
9. IgE Positive Control 1 x 1.3mL
10. IgE Negative Control 1 x 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.

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

Interpretation Of Results

OD<0.15, Negative

OD>=0.15 Positive

Precision

Intra-assay CV: ≤15%

Inter-assay CV: ≤15%

Detection Limit

Allergen: 0.35IU/mL

Total IgE: 3 IU/Ml

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