



Total IgE ELISA - pNPP

Direct enzyme-linked immunosorbent assay kit for the
quantitative determination of total serum IgE.

Cat. BL-05-E
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Summary and background of the test:

Five different types of human antibodies have been characterized : IgA-IgD-IgE-IgM-IgG.

In-vitro techniques for allergy testing have improved since the specific immunoglobulin responsible for allergic hypersensitivity was discovered and identified as IgE (1-2).

Atopic allergy is a hypersensitive immunological condition mediated by IgE (3).

Immunocompetent B-lymphocytes, if stimulated by a specific allergen, produce antibodies to the allergen.

IgE antibodies bind, via their Fc portion, to receptors on the surface of mast cells and basophilic leucocytes. Upon further stimulation, these cell-bound IgE molecules bind via their Fab portion to the allergen. This combination triggers cell degranulation and the release of various substances, including vasoactive amines.

The most common clinical manifestations of this biological process are dermatitis, rhinitis, hay fever, asthma and anaphylactic shock.

IgE determinations are most valuable in the diagnosis assessment of patients with established or suspected allergic diseases (4).

IgE values are age-related.

Some parasitic infections may also lead to increased IgE levels (5). Immunological studies of IgE myelomatosis have also been performed (6-8).

The radioactive label is still widely used in IgE determinations. Other techniques provide also adequate and reliable results: chemiluminescent immunoassays (9-10), nephelometric immunoassay (11), biotin-avidin based solid-phase (12) and radial partition fluorometric enzyme immunoassay (13).

Principle of the test :

The Bio-Line Total IgE microplates Elisa kit is a two-site enzyme-linked immunosorbent assay for the quantitative determination of IgE.

A first Mouse monoclonal antibody is immobilized onto the plastic wells.

A second polyclonal antibody is labelled with the alkaline phosphatase enzyme.

The diluted sample is incubated with the solid-phase antibody-coated well. After the incubation period, the plate is washed. Only the antigen from the patient sample remains bound onto the well.

In a second step, the conjugate-labelled antibody is added. If IgE is present in the sample a "sandwich" complex is formed:

Well---Mouse Monoclonal anti-h-IgE---sample IgE---conjugate polyclonal anti-h-IgE.

At the end of the second incubation, the wells are washed to remove unbound conjugate and other unbound components.

A third incubation is performed with the chromogen (pNPP).

The reaction is then stopped with NaOH, and the plate is read at 405 nm in a spectrophotometer.

The color intensity is directly proportional to the amount of IgE in the sample. The level of unknown IgE is then determined by comparing the optical density with data established using known IgE standards in the same assay system.

Precautions:

1. Sodium azide: Sodium Azide, used as a bacteriostatic agent, is toxic in acid medium. In addition, it may form potentially explosive lead or copper azides. To avoid dangerous deposits, waste solutions should be flushed away with large volumes of water.
2. Hepatitis and Acquired Immune Deficiency Syndrome (HTLV-III): All Bio-Line reagents included in this kit have been tested and found to be non reactive for hepatitis B surface antigen. They have also been screened and determined to be non-reactive for HTLV-III antibody. However, human serum products should be handled as if potentially capable of transmitting hepatitis, Acquired Immune Deficiency Syndrome, or other infectious agents.

Materials provided :

Kit contains sufficient reagents for 96 determinations.

1. **Total IgE standards & control:** 7 vials containing each 500 µl, except Zero 1 ml. Control: 150 ± 50 IU/ml. Standards: 0-2-5-20-50-200-500 IU/ml, calibrated against the IRP 75/502.
2. **Anti-IgE alkaline phosphatase conjugate:** 1 vial containing 10.5 ml of ready-to-use polyclonal anti-h-IgE alkaline phosphatase conjugate (blue solution).
3. **Assay buffer:** 1 vial containing 10.5 ml of ready-to-use yellow solution.
4. **Wash solution concentrate:** 1 vial of 10 ml of concentrate (blue solution), to be diluted into 500 ml of distilled water and stored at 4°C.
5. **Microplate:** 12 green color-coded strips. Break-apart wells are coated with Mouse monoclonal anti-h-IgE.
6. **Substrate diluent:** 1 vial containing 10.5 ml of pNPP diluent.
7. **Chromogen tablets:** 3 packs containing each 1 tablet of 5mg of pNPP (p-nitrophenyl phosphate).
8. **Stop solution:** 1 vial containing 10.5 ml of NaOH 1N.

Reagents provided should be stored at 2° - 8° C.

Refer to the expiration date on the kit label for stability.

Materials required but not provided:

1. Pipets, micropipets, repeating pipettors.
2. Microplate reader with 405 nm filter.
3. Incubator.
4. Horizontal shaker.
5. Microplate washer.
6. Glass vials.
7. Distilled water.
8. Microwells extra holder frame.

Specimen collection and preparation:

Sera should be separated from blood cells immediately after collection. Sera are stable for at least 7 days at 4° C and for longer periods of time when stored frozen.

Assay procedure:

Bring reagents to room temperature and mix before use. Label wells for blank, standards, control sera and unknowns.

1. Pipet 100 µl of assay buffer into each well.
2. Pipet 20 µl of standards, control and samples into the corresponding wells.
3. Cover with plastic film, homogenize at 300 rpm (horizontal shaking) and incubate all strips in an incubator for 90 minutes at 37°C.
4. Aspirate and add 300 µl of wash solution to each well. Aspirate again. Repeat twice more for a total of 3 washes/aspirations. Allow the wells to stand for 1 minute, and aspirate again.
5. Pipet 100 µl of conjugate into each well.
6. Cover with plastic film and incubate all strips in an incubator for 90 minutes at 37°C.
7. 30 minutes before step 8, prepare the chromogen-substrate solution. In an empty glass vial, dilute 1 tablet of pNPP into 3.3 ml of ready-to-use substrate diluent. Keep it **in the dark**. Use only fresh solution for each run.

8. Repeat wash procedure as described in step 4.
9. Pipet 100 µl of chromogen into each well.
10. Cover with plastic film and incubate all strips for 30 minutes at room temperature **in the dark**.
11. Pipet 100 µl of stopping solution (NaOH) into each well.
12. Homogenize at 300 rpm (horizontal shaking) and read all wells at 405 nm against the blank, set at zero.

Total IgE microplates Flow Chart.

Wells	Blank	Standards, Control	Patient samples
Reagents			
Assay buffer	-	100 µl	100 µl
Standards, control, Patient samples	-	20 µl	20 µl
Homogenize under horizontal shaking (±300 rpm), and incubate 90 min. at 37°C.			
Wash 3 x with 300 µl wash solution.			
Anti-IgE conjugate	-	100 µl	100 µl
Incubation 90 min. at 37°C.			
Prepare the chromogen (1 tab./3.3 ml substrate diluent). Wash 3 x with 300 µl wash solution.			
Chromogen	100 µl	100 µl	100 µl
Incubation 30 min. at RT in the dark .			
Stop solution	100 µl	100 µl	100 µl
1) Homogenize (300 rpm) 2) read at 405 nm			

Data table (Example).

#	Duplicate optical density		Mean OD.	%B/Bmax	IU/ml
Std 0	0.092	0.098	0.095	4.0	
Std 2	0.133	0.142	0.137	5.7	
Std 5	0.209	0.230	0.219	9.2	
Std 20	0.455	0.497	0.476	19.9	
Std 50	0.903	0.849	0.876	36.7	
Std 200	1.621	1.590	1.605	67.2	
Std 500	2.389	2.391	2.390	100	
Control	1.421	1.507	1.463	61.2	170.5
Sample 1	0.525	0.590	0.557	23.3	25.5
Sample 2	2.116	1.975	2.045	85.6	368.0

Calculation of results:

$$\% \text{ B/Bmax} = \frac{\text{Mean O.D. (Stds, Control or samples)}}{\text{Mean O.D. Std. 500}} \times 100$$

Plot % B/Bmax for each standard vs its concentration in IU/ml. The concentration of Total IgE in the unknown samples may be read directly from the standard curve.

Expected Values:

The values reported below are indicative. Each laboratory should establish its own normal range. Pediatric values are available on request.

Lower than 20 IU/ml: allergy not probable.
Between 20 and 100 IU/ml: allergy questionable.
Higher than 100 IU/ml: allergy very probable.

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