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IgE

Chemiluminescence Enzyme Immunoassay for the Quantitative Determination of IgE in Human Serum

Catalog No. C29-800

INTRODUCTION OF CHEMILUMINESCENCE IMMUNOASSAY

Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA). ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes. Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers. The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panals, such as T3, T4, TSH, Hormone panals, such as IgE, LH, FSH, and other panals. They can be used to replace conventional colorimetric ELISA that have been widely used in many research and diagnostic applications.

Introduction of IgE Immunoassay

Introduction

Patients with atopic allergic diseases such as atopic asthma, atopic dermatitis, and hay fever have been shown to exhibit increased total immunoglobulin E (IgE) levels in blood. IgE is also known as the reagenic antibody. In general, elevated levels of IgE indicate an increased probability of an IgE-mediated hypersensitivity, responsible for allergic reactions. Parasitic infestations such as hookworm, and certain clinical disorders including aspergillosis, have also been demonstrated to cause high levels of IgE. Decreased levels of IgE are found in cases of hypogammaglobulinemia, autoimmune diseases, ulcerative colitis, hepatitis, cancer, and malaria. Cord blood or serum IgE levels may have prognostic value in assessing the risk of future allergic conditions in children. Certain groups of white blood cells, including basophils and tissue mast cells, have membrane receptors for the IgE molecule. These target cells, through a series of complex reactions, form a combination of a specific allergen with antibody-sensitized basophils such as histamine, into the blood stream. As a result of these biochemical mediators, there is a constriction of smooth muscles, dilation of small blood vessels, activation of blood platelets, and irritation of skin nerve endings characteristic of allergic reactions. Typical clinical symptoms of immediate hypersensitivity areinflammation and itching in a skin reaction, or congestion in a bronchial reaction. The IgE serum concentration in a patient is dependent on both the extent of the allergic reaction and the number of different allergens to which he is sensitized. Nonallergic normal individuals have IgE concentrations that vary widely and increase steadily during childhood, reaching their highest levels at age 15 to 20, and thereafter remaining constant until about age 60, when they slowly decline. The IgE Quantitative Enzyme Immunoassay provides a rapid, sensitive, and reliable assay for total serum IgE. The minimal sensitivity of this assay is about 5.0 IU/ml.

Principle of the test

Immunospec IgE Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-IgE antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-IgE antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the IgE antibody coated microtiterwells and incubated. If IgE is present in the specimen, it will combine with the antibody on the well, and then IgE antibody labeled with horseradish peroxidase (conjugate) are added. The conjugate will bind immunologically to the IgE on the well, resulting in the IgE molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a incubation at room temperature, the wells are washed with wash buffer to remove unbound labeled antibodies. A solution of chemiluminescent substrate is added and then read relative light units (RLU) in the appropriate Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of IgE in the sample. By reference to a series of IgE standards assayed in the same way, the concentration of IgE in the unknown sample is quantified.

Materials and Components

Materials provided with the test kit:

1. Antibody-coated microtiter wells. 96 wells per bag.
2. Zero Buffer, 12 ml.
3. Reference standard set, contains 0, 10, 50, 100, 400, and 800 IU/ml IgE, liquid.
4. Enzyme Conjugate Reagent, 12 ml.
5. 20x Wash Buffer, 30 ml
6. Chemiluminescence Reagent A, 6.0 ml.
7. Chemiluminescence Reagent B, 6.0 ml

Materials required but not provided:

- Precision pipettes: 20µl~200µl.
- Disposable pipette tips.
- Distilled water.
- Glass tubes or flasks to mix Chemiluminescence Reagent A and Chemiluminescence Reagent B.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate Luminometer

Specimen Collection and Preparation

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

Storage of Test Kit and Instrumentation

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. To prepare substrate solution, make an 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
3. Prepare the washing solution by diluting 1 part of the 20X PBS concentrate to 19 parts of distilled water.

Assay procedures

1. Secure the desired number of coated wells in the holder.
2. Dispense 20µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of Zero Buffer into each well.
4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the microtiter wells 5 times with running tap or distilled water.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150µl of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 30 minutes.
11. Remove the incubation mixture by flicking plate contents into a waste container.
12. Rinse and flick the microtiter wells 5 times with wash buffer.
13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
14. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
15. Read wells with a chemiluminescence microwell reader 5 minuters later. (between 5 and 20 min. after dispencing the substrates).

Important Note:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. If there are bobbles existing in the wells, the false readings will be created. Please use distilled water to remove the bobbles before adding the substrate.

Calculation of Results

1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend use proper software to calculate the results. The best curve fitting used in the assays are quadratic regression or 4-parameter regression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against IgE concentration in IU/ml on linear graph paper, with RLU on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgE in IU/ml from the standard curve.

Example of Standard Curve (Black wells)

Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is runing at the same time

IgE (IU/ml)	RLU (104)
0	0.020
10	0.133
50	0.812
100	2.162
400	4.973
800	11.44

Expected values and sensitivity

The total IgE level in a normal, allergy-free adult is less than 150 IU/ml of serum. The minimum detectable concentration of IgE by this assay is estimated to be 5.0 IU/ml.

References

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