



## DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F,  
Calabasas, CA 91302  
Tel: (818) 591-3030 Fax: (818) 591-8383

[onestep@rapidtest.com](mailto:onestep@rapidtest.com)

[technicalsupport@rapidtest.com](mailto:technicalsupport@rapidtest.com)

[www.rapidtest.com](http://www.rapidtest.com)

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# IgE

## CHEMILUMINESCENCE ENZYME IMMUNOASSAY (CLIA) HUMAN CHORIONIC GONADOTROPIN (IgE)

Cat # 9050-16

### Chemiluminescence Enzyme Immunoassay for the Quantitative Determination of HUMAN CHORIONIC GONADOTROPIN (IgE) in Human Serum

#### 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 panels, such as T3, T4, TSH, Hormone panels, such as IgE, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISA that have been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

The CLIA Kits have been validated on the **MPL2** microplate luminometer from Berthold Detection System, **Lus2** microplate luminometer from Anthos, **Centro LB960** microplate luminometer from Berthold Technologies, and **Platelumino** from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

## 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, u higob53 TD.

## 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. 50x Wash Buffer, 15 ml
6. Chemiluminescence Reagent A, 6.0 ml.
7. Chemiluminescence Reagent B, 6.0 ml.

### **Materials required but not provided:**

- Precision pipettes: 20 $\mu$ l~200 $\mu$ l.
- 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. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

## Assay procedures

1. Secure the desired number of coated wells in the holder.
2. Dispense 20 $\mu$ l of standard, specimens, and controls into appropriate wells.
3. Dispense 100 $\mu$ l 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 washing buffer(1X).
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 washing buffer(1X).
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 minutes later. (between 5 and 20 min. after dispensing 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 running at the same time.

IgE (IU/ml)	RLU (10 <sup>4</sup> )
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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Tel: (818) 591-3030 Fax: (818) 591-8383

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