



DIAGNOSTIC AUTOMATION, INC.

21250 Califa Street, Suite 102 and 116, Woodland Hills, CA 91367

Tel: (818) 591-3030 Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com



96 tests



9005 -16

Chemiluminescence Immunoassay Enzyme Immunassay (CLIA)

TSH

REF 9005-16

Chemiluminescence Enzyme Immunoassay for the Quantitative Determination of Thyroid Stimulating Hormone (TSH) 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 hCG, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISAs that have been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, chemiluminescence immunoassay will play an important part in the diagnostic and research areas that ELISAs cannot 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 TSH IMMUNOASSAY

The determination of serum or plasma levels of thyroid stimulating hormone (TSH) is recognized as a sensitive method in the diagnosis of primary and secondary hypothyroidism. TSH is secreted by the anterior lobe of the pituitary gland and induces the production and release of thyroxine and triiodothyronine from the thyroid gland. It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta. Although the concentration of TSH in the blood is extremely low, it is essential for the maintenance of normal thyroid function. The release of TSH is regulated by a TSH-releasing hormone (TRH) produced by the hypothalamus. The levels of TSH and TRH are inversely related to the level of thyroid hormone. When there is a high level of thyroid hormone in the blood, less TRH is released by the hypothalamus, so less TSH is secreted by the pituitary. The opposite action will occur when there is decreased thyroid hormone in the blood. This process is known as a negative feedback mechanism and is responsible for maintaining the proper blood levels of these hormones. TSH and the pituitary glycoprotein, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG), have identical alpha chains. The beta chain is distinct but does contain identical amino acid sequences, which can cause considerable cross-reactivity with some polyclonal TSH antisera. The use of a monoclonal antibody in this TSH EIA test eliminates this interference, which could result in falsely elevated TSH values in either menopausal or pregnant females--a population whose evaluation of thyroid status is clinically significant.

PRINCIPLE OF THE TEST

The TSH CLIA test utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase (microtiter wells) immobilization and a goat anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60 minute incubation at room temperature, the wells are washed 5 times by wash solution to remove unbound anti-TSH conjugate. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in a luminometer. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of TSH in the sample. By reference to a series of TSH standards assayed in the same way, the concentration of TSH 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. Set of Reference Standards: 0, 0.5, 2, 5, 10, 20 and 40 μ IU/ml, liquid, ready for use.
3. Enzyme Conjugate Reagent, 12 ml.
4. 50x Wash Buffer Concentrate, 15 ml
5. Chemiluminescence Reagent A, 6.0 ml.
6. Chemiluminescence Reagent B, 6.0 ml.
7. Control Set (optional)

Materials required but not provided:

- Precision pipettes: 40 μ l~200 μ l and 1.0 ml.
- 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.
- 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 brought to room temperature (18-25°C) before use.
2. To prepare Chemiluminescence Substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Discard the excess after use.
3. Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, dilute 15 ml of Wash Buffer Concentrate (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this step.
5. Incubate at room temperature (18-25°C) for about 60 minutes.
6. Rinse and flick the microtiter wells 5 times with washing buffer (1X).
7. Strike the wells sharply onto absorbent paper to remove residual water droplets.
8. Dispense 100 µl of Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
9. 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 RLU readings.
2. If there are bubbles existing in the wells, false readings will be created. Please use distilled water to remove the bubbles 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 using proper software to calculate the results. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against

TSH concentration in $\mu\text{IU/ml}$ on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

- Using the mean absorbance value for each sample, determine the corresponding concentration of TSH in $\mu\text{IU/ml}$ from the standard curve.

EXAMPLE OF STANDARD CURVE

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 to run the 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.

TSH ($\mu\text{IU/ml}$)	Relative Light Units (RLU) (10^5)
0	0.001
0.5	0.08
2	0.49
5	1.54
10	3.11
20	6.22
40	10.56

EXPECTED VALUES AND SENSITIVITY

The mean TSH values based on 160 random normal adult blood samples, is 1.6 (0.4-7.0) $\mu\text{IU/ml}$. The minimum detectable concentration of TSH by this assay is estimated to be 0.02 $\mu\text{IU/ml}$.

LIMITATIONS OF PROCEDURE

There are some limitations of the assay.

- As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
- Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background reading.

REFERENCES

- Rongen HA, Hoetelmans RM, Bult A, van Bennekom WP. Chemiluminescence and immunoassays. **J Pharm Biomed Anal** 1994 Apr;12(4):433-62.
- Koszegi T, Immunoluminometric detection of human procalcitonin. **J Biochem Biophys Methods** 2002 Oct;53(1-3):157-64.
- Roda A, Simoni P, Mirasoli M, Baraldini M, Violante FS. Development of a chemiluminescent enzyme immunoassay for urinary 1-hydroxypyrene **Anal Bioanal Chem** 2002 Apr;372(7-8)
- Soos M. and Siddle K. **J. Immunol. Methods** 1982; 51: 57-68
- Wada H.G., Danisch R.J. and Baxter S.R. **Clin. Chem.** 1982; 28: 1862-1866
- Uotila M., Ruoslahti E. and Engvall E. **J. Immunol. Methods** 1981; 42: 11-15

7. Burger H.G. and Patel Y.C. Thyrotropin releasing hormone-TSH **Clinic. Endocrinol. and Metab.** 1977; 6: 831
8. Snyder P.J. and Utiger R.D. **J. Clin. Endocrinol. Metab.** 1972; 34: 380-385

Date Adopted	2016-05-03
REF 9005-16	DA-TSH-INTERNATIONAL

 **DIAGNOSTIC AUTOMATION, INC.**

21250 Califa Street, Suite 102 and 116, Woodland Hills, CA 91367

Tel: (818) 591-3030 Fax: (818) 591-8383

ISO 13485-2003



Revision Date: 2016-04-01