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$\Sigma=96$ tests

REF

9043-16

CHEMILUMINESCENCE
ENZYME IMMUNOASSAY (CLIA)
FREE BETA-SUBUNIT OF HUMAN
CHORIONIC GONADOTROPIN (Free β -hCG)

Free Beta hCG

REF

9043-16

Chemiluminescence Enzyme Immunoassay for the Quantitative Determination of Human Chorionic Gonadotropin (Free β -hCG) 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 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 FREE BETA hCG IMMUNOASSAY

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone normally produced by placenta during pregnancy. The hormone is present in blood and urine around seven to thirteen days following implantation of the fertilized ovum. Structurally intact hCG molecules consist of two non-covalently linked polypeptide subunits, the alpha and beta chain subunits. Measurement of intact hCG and of the alpha subunit of hCG appears to give similar results in blood and urine but not the levels of beta subunit. In the normal second-trimester maternal sera, the level of intact hCG range from 20,000 mIU/ml to 50,000 mIU/ml. In contrast, the levels of either free α - or free β -hCG are on average one half of 1% of hCG levels. hCG and the free subunits appear not to be useful as serological markers for nontrophoblastic tumors; however, the absolute increase of β -hCG level in choriocarcinoma patients clearly differentiates it from normal pregnancy.

Recent studies showed a significant increase in the level of free β -hCG subunit in trisomy 21 cases as compared with controls. Hence, it has been suggested that free β -hCG subunit assay in a combination of maternal serum AFP could be effective in a screening protocol for trisomy 21.

PRINCIPLE OF THE TEST

The Free β -hCG Quantitative Test Kit is based on a solid phase Chemiluminescence enzyme-linked immunosorbent assay. The assay system utilizes one monoclonal anti- Free β -hCG antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti- Free β -hCG antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the FREE BETA HCG antibody coated microtiter wells. Then Free β -hCG antibody labeled with horseradish peroxidase (conjugate) is added. If human Free β -hCG is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the Free β -hCG molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 1 hour incubation at room temperature, the wells are washed with water 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 Free β -hCG in the sample. By reference to a series of Free β -hCG standards assayed in the same way, the concentration of β -hCG in the unknown sample is quantified.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

1. Antibody-coated microtiter plate with 96 wells.
2. Reference standards, 0, 2.5, 5, 10, 25, 50 mIU/ml, in the sample diluent against WHO IRP 75/551. (1 mIU/ml = 1 mIU/ml for β -hCG). Lyophilized standards, reconstitute with 0.5 ml distilled water each before use.

3. Zero Buffer (Sample diluent), 20 ml
4. Enzyme Conjugate Reagent, 18 ml
5. 50x Wash Buffer Concentrate, 15 ml
6. Chemiluminescence Reagent A, 6.0 ml.
7. Chemiluminescence Reagent B, 6.0 ml.

Materials required but not provided:

- Precision pipettes: 50 μ l~200 μ l, 1.0ml
- 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

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, and mixed by gently inverting or swirling prior to use. Do NOT induce foaming.
2. Add 0.5 ml of distilled water to reconstitute the lyophilized standards. Allow the reconstituted materials to stand for at least 20 minutes. Mix gently. The reconstituted standards should be stored sealed at 2-8 °C.
3. To prepare substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
4. 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 50 μ l of standard, specimens, and controls into appropriate wells.
3. Dispense 100 μ l of Zero Buffer into each well.
4. Thoroughly mix for 10 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at 37°C for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a sink.
7. Rinse and flick the microtiter wells 5 times with washing solution.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150 μ l of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.

10. Incubate at 37°C for 30 minutes. Remove the incubation mixture by flicking plate contents into sink.
11. Rinse and flick the microtiter wells 5 times with washing solution.
12. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
13. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
14. Read wells with a Luminometer 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 bubbles existing in the wells, the 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. 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 Free Beta HCG concentration in mIU/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 FREE BETA HCG in mIU/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 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.

FREE BETA HCG (mIU/ml)	RLU (10 ⁵)
0	0.11
2.5	1.22
5.0	2.47
10.0	5.73
25.0	16.62
50.0	32.31

EXPECTED VALUES AND INDICATIONS FOR QUANTITATIVE FREE β-hCG ASSAY::

1. In early pregnancy, free β-hCG concentration was found 10~80 mIU/ml. The free β-hCG /intact hCG ratio was found 3.08~`3.28 percents. After 6 to 7 weeks the free β-hCG and the ratio value declined. During the second and third trimester, a constant ratio was observed about 1 percent.
2. Serum samples from 40 normal subjects were assayed, in this population, 99% of the values were less than 0.4 mIU/ml. Serum hCG and free subunit levels in sera from patients with gestational choriocarcinoma were reported as following: (Ozuturk et al. Endocrinology, 1987)

Patient No	HCG (mIU/ml)	α -hCG (mIU/ml)	β -hCG(mIU/ml)
1	210,000	112	8,000
2	22,195	20	1,300
3	6,840	1	232
4	36,000	44	3,900
5	4,200	2	350

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