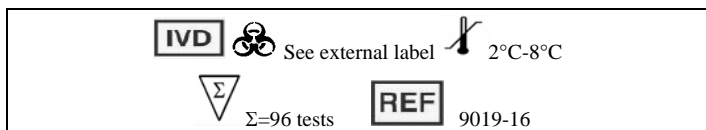



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Ferritin

CAT No: 9019-16

Chemiluminescence Immunoassay

Chemiluminescence Enzyme Immunoassay for the Quantitative Determination of Ferritin 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, FERRITIN, 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 FERRITIN IMMUNOASSAY

One of the most prevalent disorders of man is the dietary deficiency of iron and the resulting anemia. Therefore, the assays of iron, total iron binding capacity and other assessments of iron compounds in the body are clinically significant.

Iron-storage compounds in the body include hemoglobin, hemosiderin, myoglobin and the cytochromes. In most tissues, ferritin is a major iron-storage protein. Human ferritin has a molecular weight of approximately 450,000 daltons, and consists of a protein shell around an iron core; each molecule of ferritin may contain as many as 4,000 iron atoms. Under normal conditions, this may represent 25% of the total iron found in the body. In addition, ferritin can be found in several isomers.

High concentrations of ferritin are found in the cytoplasm of the reticuloendothelial system, the liver, spleen and bone marrow. Methods previously used to measure iron in such tissues are invasive, cause patient trauma and lack adequate sensitivity.

The measurement of ferritin in serum is useful in determining changes in body iron storage, and is non-invasive with relatively little patient discomfort. Serum ferritin levels can be measured routinely and are particularly useful in the early detection of iron-deficiency anemia in apparently healthy people.

Serum ferritin measurements are also clinically significant in the monitoring of the iron status of pregnant women, blood donors, and renal dialysis patients. High ferritin levels may indicate iron overload without apparent liver damage, as may be noted in the early stages of idiopathic hemochromatosis. Ferritin levels in serum have also been used to evaluate clinical conditions not related to iron storage, including inflammation, chronic liver disease, and malignancy.

The *Ferritin Enzyme Immunoassay Test Kit* provides a rapid, sensitive and reliable assay. The antibodies developed for the test will determine a minimal concentration of human ferritin of 5 ng/ml. There is minimal cross-reactivity with human serum albumin, alpha-fetoprotein, human hemoglobin, human transferrin, and ferric chloride.

Principle of the Test

The FERRITIN CLIA test utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact FERRITIN molecule. Mouse monoclonal anti-FERRITIN antibody is used for solid phase (microtiter wells) immobilization and a goat anti-FERRITIN 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 FERRITIN molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60 minutes incubation at room temperature, the wells are washed 5 times by wash solution to remove unbound anti-FERRITIN conjugate. A solution of

chemiluminescent substrate is then added and read relative light units (RLU) in a Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of FERRITIN in the sample. By reference to a series of FERRITIN standards assayed in the same way, the concentration of FERRITIN 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, 10, 50, 100, 400, and 800 ng/ml, Liquid, Ready for use.
3. Enzyme Conjugate Reagent, 12 ml.
4. 20x Wash Buffer, 30 ml
5. Chemiluminescence Reagent A, 6.0 ml.
6. Chemiluminescence Reagent B, 6.0 ml.

Materials required but not provided:

- Precision pipettes: 40µl~200µl 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.
- Microtiter plate reader.

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 (18-22°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. Prepare the washing solution by diluting 1 part of the 20X PBS concentrate to 19 parts of distilled water.

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 (18-22°C) for about 60 minutes.
6. Rinse and flick the microtiter wells 4 times with washing solution and final 1 time with distilled water.
7. Strike the wells sharply onto absorbent paper to remove residual water droplets.
8. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
9. Read wells with a chemiluminescence microwell reader 15 minutes later. (between 10 and 20 min. after dispensed the substrates).

Important Note:

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Calculation of Results

1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend to use a 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 FERRITIN concentration in ng/ml on linear graph paper, with absorbance 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 FERRITIN in uIU/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.

FERRITIN (ng/ml)	Relative Light Units (RLU) (10 ⁵)
0	0.05
10	0.34
50	1.98
100	3.08
400	10.22
800	19.23

Expected Values and Sensitivity

Each laboratory must establish its own normal ranges based on patient population. The results provided below are based on a limited number of healthy adult blood specimens. The minimal sensitivity of the test is 5.0 ng/ml.

	Male	Female
Number	80	90
Mean (ng/ml)	170.0	71.0
Mean (ng/ml)	32.0-501.0	3.5-223.5

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