FERRITIN ENZYME IMMUNOASSAY TEST KIT
Catalog Number: OKBA00001

Aviva Systems Biology
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Enzyme Immunoassay for the Quantitative Determination of Ferritin in Human Serum

FOR RESEARCH PURPOSES ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

PRINCIPLE OF THE ASSAY
The Aviva Ferritin Quantitative Test is based on the principle of a solid phase enzyme-linked immunosorbent assay.[12,13,14] The assay system utilizes rabbit anti-ferritin for the solid phase (microtiter wells) immobilization and mouse monoclonal anti-ferritin in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of 3,3',5,5'-Tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of ferritin is directly proportional to the color intensity of the test sample.

REAGENTS AND MATERIALS PROVIDED
1. *Antibody-Coated Wells (1 plate, 96 wells)*
   Microtiter wells coated with rabbit anti-ferritin.
2. *Enzyme Conjugate Reagent (13 mL)*
   Contains mouse monoclonal anti-ferritin conjugated to horseradish peroxidase.
3. *Reference Standard Set (0.5 mL/vial)*
   Contains 0, 15, 80, 250, 500, and 1,000 ng/mL human liver or spleen ferritin in bovine serum with preservatives. 0.5 mL each, liquid, ready to use.
4. *TMB Reagent (One-Step) (1 bottle, 11 mL)*
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
5. *Stop Solution (1N HCl) (1 bottle, 11 mL)*
   Contains diluted hydrochloric acid.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Distilled or deionized water
2. Precision pipettes: 0.02, 0.05, 0.1, 0.2 and 1 mL
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450nm.
5. Vortex mixer, or equivalent
6. Absorbent paper
7. Graph paper
8. Quality control material (e.g., BioRad Lyphochek Control sera)

WARNINGS AND PRECAUTIONS
1. **CAUTION:** This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[21]
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.

STORAGE CONDITIONS
1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

SPECIMEN COLLECTION AND PREPARATION
1. 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. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.
3. Specimens with expected values greater than 1,000 ng/ml (e.g. dialysis samples) should be diluted with Zero Standard prior to assaying. A 1:10 initial dilution is recommended.

INSTRUMENTATION
A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Samples with expected values greater than 1000 ng/mL should be diluted with Zero Standard prior to assaying. A 1:10 initial dilution is recommended.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 20µL of standards, samples, and controls into appropriate wells.
3. Dispense 100µL of Enzyme Conjugate Reagent into each well.
4. Gently mix for 30 seconds. It is very important to have complete mixing.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking well contents into a suitable waste container.
7. Rinse the wells 5 times with distilled or dionized water. (Please do not use tap water.)
8. Strike the wells sharply on absorbent paper to remove residual water droplets.
9. Dispense 100 µL TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature, in the dark, for 20 minutes.
11. Stop the reaction by adding 100µL of Stop Solution (1N HCl) into each well.
12. Gently mix for 5 seconds.
13. Read OD at 450nm with a microtiter well reader ***within 15 minutes.***

Calculation of Results

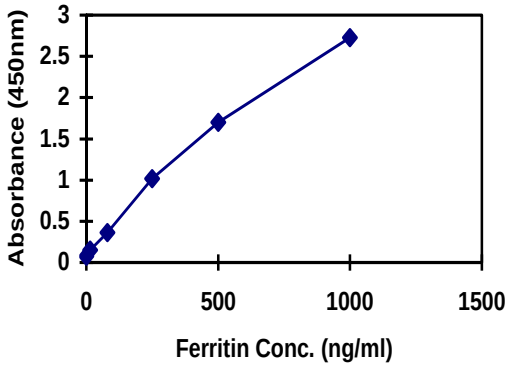
1. Calculate the mean absorbance value (OD_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of ferritin in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further converted by the appropriate dilution factor.

Example of Standard Curve

1. Results of a typical standard run of the assay are shown below.

Ferritin (ng/mL)	Absorbance (450 nm)
0	0.074
15	0.150
80	0.362
250	1.017
500	1.699
1000	2.728

2. This standard curve is for illustration only, and should not be used to calculate unknowns.



Technical Consultation

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