Ferritin Test System  
Product Code: 2825-300

1.0 INTRODUCTION
Intended Use: The Quantitative Determination of Circulating Ferritin Concentrations in Human Serum by a Microparticle Immunoenzymometric assay

2.0 SUMMARY AND EXPLANATION OF THE TEST
Ferritin, in circulation, as measured in serum levels is a satisfactory index of body's iron storage. The iron storage is directly measured by the hepatic iron storage, iron absorption studies, liver biopsies and microscopic examinations of bone marrow aspirates. Laboratory tests are defined as examinations of blood and iron overload (Hemochromatosis) are conditions associated with body's iron storage or lack thereof. Measurements of total iron binding capacity (TIBC) have widely been used as aids in the determination of these conditions. However, an assay of ferritin is simply more sensitive and reliable means of demonstration thereof.

Ferritin is present in blood in very low concentrations. Normally, approximately 1% of plasma iron is contained in Ferritin. The plasma ferritin, is in equilibrium with body stores, and variations of iron storage. The plasma concentrations of ferritin decline very early in anemic conditions like development of iron deficiency, long before the changes are observed in the blood hemoglobin concentration, size of the erythrocytes and TIBC. Thus, measurements of serum ferritin can serve as an early indicator of iron deficiency that is uncomplicated by other concurrent conditions. At the same time a large number of chronic conditions can result in elevated levels of serum ferritin. These include chronic infections, chronic inflammatory diseases such as rheumatoid arthritis, heart disease and some other malignancies, especially lymphomas, leukemia, breast cancer and neuroblastoma. In patients who have these chronic disorders together with iron deficiency, serum ferritin levels are often normal. An increase in circulating ferritin is observed in patients with viral hepatitis or after anoxia or toxemia as a result of pernicious anemia. Ferritin is present in liver cells. Elevated serum ferritin levels are found in patients with hemochromatosis and hemosiderosis.

Circulating ferritin levels have been used by clinicians, as an aid, in the diagnosis of the diseases listed above. Ferritin has been proved as a valuable tool in differential diagnosis of anemia due to iron deficiency and anemia due to other disorders and, in exposing the depletion of iron stores. Since no known test can confirm absence of inflammatory agents that are absent, all hemoglobin based products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control and Prevention's "Laboratory and Blood Bank best practices for Blood Collection, Processing, and Storage of Blood, Plasma, Platelets, and Blood Components". All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all hemoglobin based products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control and Prevention’s "Laboratory and Blood Bank best practices for Blood Collection, Processing, and Storage of Blood, Plasma, Platelets, and Blood Components”.

2.1 Materials Provided:
A. Ferritin Calibrators – 1ml / vial - Icons A-F
B. Ferritin Biotin Reagent – 13ml/vial - Icon B
C. Ferritin Enzyme Reagent – 13ml/vial - Icon C
D. Streptavidin Coated Plate – 96 wells - Icon D
E. Wash Solution Concentrate – 20 ml - Icon E
F. Substrate A – 7ml/vial - Icon F
G. Substrate B – 7ml/vial - Icon G
H. Stop Solution – 8ml/vial - Icon H
I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Do not use reagents that are contaminated or have bacterial growth.

2.2 Procedure

Before proceeding with the assay, bring all reagents, sera and controls to room temperature (20 - 27°C). The procedure should be performed by a skilled individual or trained professional. The specimens shall be blood; serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without anticoagulants or additive(s). Allow the blood to clot for 3 hours. Centrifuge the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the sample(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repeated freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

2.2.1 Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. Prepare the Working Substrate Solution. Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly.

2.2.2 Wash Solution
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In the present procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-ferritin antibody. Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. Simultaneously another reactant, labeled antibody, binds to the streptavidin coated on the microwells resulting in immobilization of the complex. The reaction is illustrated by the following equation:

\[ \text{Ag(Ferritin)} + \text{BtnAb(m)} \rightarrow \text{Antigen-Antibody complex (Variable Quan.)} \]

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Do not use reagents that are contaminated or have bacterial growth.

6.0 REAGENT PREPARATION:

1. Wash Buffer: Dilute contents of wash solution to 1000 ml with distilled or deionized water in a suitable storage container. Store at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

7.0 QUALITY CONTROL
Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be used in every test performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can be interpreted as indication of improper mixing or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, sera and controls to room temperature (20-27°C). The procedure should be performed by a skilled individual or trained professional.

1. Pipette capable of delivering 25, 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 ml and 0.350 ml volumes with a precision of better than 1.5%.
3. Microplate washers (optional).
4. Microplate Reader with 450nm and 690nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.

5.0 PRECAUTIONS
For In Vitro Diagnostic Use Not for Internal or External Use in Humans or Animals

6.0 SPECIMEN COLLECTION AND PREPARATION
The specimens shall be blood; serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without anticoagulants or additive(s). Allow the blood to clot for 3 hours. Centrifuge the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the sample(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

7.0 QUALITY CONTROL
Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be used in every test performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can be interpreted as indication of improper mixing or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION:

1. Wash Buffer: Dilute contents of wash solution to 1000 ml with distilled or deionized water in a suitable storage container. Store at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

7.0 QUALITY CONTROL
Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be used in every test performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can be interpreted as indication of improper mixing or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.
12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc. -

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.

2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.

3. Highly lipemic or grossly contaminated specimen(s) should not be used.

4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.

5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.

6. Plate readers measure vertically. Do not touch the bottom of the wells.

7. Failure to remove adhering solution adequately in the aspiration or decantation step(s) may result in poor replication and spurious results.

8. Use components from the same lot. No intermixing of reagents from different batches.

9. Accurate and precise pipetting, as well as following the exact protocol and repeating the dose response curve at (154 ng/ml) ferritin concentration (See Figure 1).

10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compatibility and proper device usage.

11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventive maintenance.

12. Risk Analysis as required by CE Mark IV Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.

2. Laboratory results alone are only one aspect for determining the concentration of ferritin in unknown specimens. Therefore, the laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the technicians using the method with a population indigenous to the area in which the laboratory is located.

13.0 EXPECTED RANGE OF VALUES

Approximate reference ranges for normal males and females adults were established by using 400 normal sera with the Ferritin AccuBind™ ELISA test system.

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-220 ng/ml</td>
<td>10-124 ng/ml</td>
</tr>
</tbody>
</table>

In addition to the above the following ranges were assigned based on the available literature. However, these ranges were confirmed using AccuBind™ Ferritin Microplate ELISA Procedure with limited number of samples.

14.0 HIGH DOSE EFFECT

It is important to keep in mind that any normal range establishment is dependent upon a multiplicity of factors like the specificity of the method, the locale, the population tested and the precision of the method in the hands of technicians. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the technicians using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the ferritin AccuBind™ ELISA system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) of each of these control sera are presented in Table 1 and Table 3.

**TABLE 2**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>43.5</td>
<td>1.6</td>
<td>3.1%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>110.5</td>
<td>6.1</td>
<td>5.5%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>349.6</td>
<td>7.5</td>
<td>2.2%</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

**TABLE 3**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>10</td>
<td>41.2</td>
<td>2.3</td>
<td>5.5%</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
<td>113.2</td>
<td>8.1</td>
<td>7.2%</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>372.4</td>
<td>11.8</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The minimum detectable dose (Sensitivity) is defined as the lowest concentration (2 σ) of the mean absorbance for twenty replicates for zero calibrator. 2 σ of the mean absorbance for twenty replicates for zero calibrator, 2 σ of the mean absorbance for twenty replicates for zero calibrator.

14.3 Specificity

The cross-reactivity of the ferritin AccuBind™ ELISA test system to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a neto between dose of interfering substance to dose of Ferritin needed to produce the same absorbance.

**TABLE 4**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Ferritin</td>
<td>100%</td>
</tr>
<tr>
<td>Spleen Ferritin</td>
<td>100%</td>
</tr>
<tr>
<td>Human Heart Ferritin</td>
<td>&lt;1.0%</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>&lt;0.1%</td>
</tr>
</tbody>
</table>

15.0 REFERENCES


