Ferritin, in circulation, as measured in serum levels is a satisfactory index of body’s iron storage. The iron storage is directly measured by quantitative phlebotomy, iron absorption studies, liver biopsies and immunosorbent examinations of bone marrow aspirates. Iron deficiency (Anemia) 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 these disorders.

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 iron and variations of iron storage. The plasma concentrations of ferritin decline very early in anemia. Ferritin levels may be measured for iron deficiency long before the changes are observed in the blood hemoglobin concentration, size of the erythrocytes and TIBC. Therefore, measurements of serum ferritin levels serve as an early indication of iron deficiency that is uncomplicated by other concurrent conditions. At the same time a large number of chronic conditions (e.g. cancer, infections, inflammation) have as a result elevated serum ferritin levels. These include chronic infections, inflammatory diseases such as rheumatoid arthritis, heart disease and some other malignancies, especially lymphomas, leukemias, breast cancer and neuroblastoma. In patients who have these chronic disorders the ferritin levels are measured as a tumor marker. An increase in circulating ferritin is observed in patients with viral hepatitis or after a toxic liver injury as a release of ferritin from the injured 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 several other disorders. It has proved as a valuable tool for the diagnosis of liver disease due to iron deficiency and anemia due to other disorders and, in exposing the depletion of iron reserves long before the onset of anemia. Specific conditions (e.g. infections) have been linked with the erosion of iron storage during pregnancy and in patients undergoing dialysis. Serum ferritin is routinely used as a screen for iron deficiency for a variety of populations like blood donors and people who are receiving regular blood transfusions or iron replacement therapy.

In this method, ferritin calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for ferritin) is added and the reactants mixed. Reaction results between the biotinylated ferritin antibody and native ferritin to form an immune complex that is deposited on the streptavidin coated well. The excess serum proteins are washed away via a wash step. Another ferritin specific antibody, labeled with an enzyme, is added to the wells. The enzyme labeled antibody binds to the ferritin already immobilized on the well. Excess enzyme is washed off via a wash step. A light signal is generated by the addition of a substrate. The intensity of the light generated is directly proportional to the concentration of the ferritin in the sample.

The employment of several serum references of known ferritin levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with ferritin concentration.

### 3.0 PRINCIPLE

Immunoenzymometric sequential assay (Type 4):

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 this procedure, the immobilization takes place during the assay at the surface of a microplate well forming an immobilized Antigen-Antigen complex. Simultaneously the biotin attached to the antibody binds to the streptavidin coated on the microwells resulting in immobilization of the complex. The interaction is illustrated by the following equation:

\[
\text{Anti-Ag} + \text{Ag-Ab bound to the well} \rightarrow \text{Immobilized complex (IC)} \\
\]

A suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled Antibody of Washed Antigen-Complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce light measurable with an enzyme-labeled luminesometer. The enzyme activity on the well is directly proportional to the native free antigen concentration. By utilizing several different serum references the assay can be rendered a dose response curve. The concentration of ferritin can be calculated from the dose response curve.

### 5.0 PRECAUTIONS

- **Not for Internal or External Use in Humans or Animals**
- 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 human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory practice, especially aseptic technique and the use of appropriate personal protective equipment should be observed. It is very important to dispense all reagents close to the bottom of the coated well.
- Discard all solutions and reagents in plastic containers to avoid aerosol exposure. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to the bottom of the coated well. Do not use any unused microwell strips back into the plastic wrap or microwell cover for incubation steps.
- Discard the contents of the microplate by decantation or aspiration. Transfer any remaining serum to a new collection container and store at 2-8°C.
- The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. Use only blood collected in a suitable container. Store diluted buffer at 2-30°C for up to 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 use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05ml of the sample is required.

### 6.0 SPECIMEN COLLECTION AND PREPARATION

- **Wash Buffer**
  - Dilute contents of Wash Concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-8°C for up to 36 hours mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

### 7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure 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 indicate unnoticed changes in experimental conditions or degradation of reagents. Fresh reagents should be used to determine the reason for the variations.

### 8.0 REAGENT PREPARATION

1. **Wash Buffer**
   - Dilute contents of Wash Concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-8°C for up to five (5) days.

2. **Working Signal Reagent Solution** - Store at 2 - 8°C.
   - Determine the amount of reagent needed and prepare by mixing with a reference of known concentration.

3. **Ferritin Biotin Reagent** - Store B in a clean container. For example, add 0 ml of A and 1 ml of B (or 1 ml of solution if B was made). Discard the unused portion if not used within 36 hours mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

4. **Product Insert**
   - Not do use reagents beyond the kit expiration date.

5. **Note 2:** Avoid extended exposure to light. Only reagents that are stable for sixty (60) days at 2-8°C. Kit and component stability are identified on the label.

### 9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).

**Test procedure should be performed by a skilled individual or trained professional!**

1. Format the microplates' wells for each serum reference, control and specimen.
2. Pipette 0.025 ml (25 µl) of the appropriate serum reference, control or specimen into the well.
3. Add 0.100 ml (100 µl) of the Ferritin Biotin Reagent to each well.
4. After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration.
5. Add 0.100 ml (100 µl) of the Ferritin Tracer Reagent to each well.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Pipette 0.025 ml (25 µl) of the appropriate serum reference, control or specimen into the well.
8. Add 0.100 ml (100 µl) of the Ferritin Reagent Tracer to each well.
9. It is very important to dispense all reagents close to the bottom of the coated well.
10. Discard the contents of the microplate by decantation or aspiration.
11. Add 350 µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. An automatic or manual plating station can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to the bottom of the coated well. Wash. Decant the wash and repeat four (4) additional times.
12. Add 0.100 ml of Ferritin Tracer Reagent. It is very important to avoid air bubbles. Avoid aerosol exposure. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to the bottom of the coated well.
11.0. Q.C. PARAMETERS
In order for the assay results to be considered valid the following criteria should be met:
1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS
The MSDS 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 degradation.
3. Highly lipemic, hemolyzed 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 signal reagent initiates a kinetic reaction, therefore the signal station(s) should be added in the same sequence to eliminate any time-degradation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermingling of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.
9. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
10. 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 preventative maintenance.
11. Risk Analysis as required by CE Mark IVDD 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 patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incompletely interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. Serum ferritin contains 20-25% iron: its concentration is a good measure of iron stores in normal persons and individuals with iron deficiency. Ferritin levels <10 ng/ml usually indicate iron deficiency anemia. Levels over 250 ng/ml would normally indicate hemochromatosis caused by certain liver diseases. Fasting, acute leukemia, inflammatory diseases, regular heavy alcohol intake and some other liver inflammations could cause elevated plasma ferritin levels as well.

13.0 EXPECTED RANGES OF VALUES
Approximate reference ranges for normal males and female adults were established by using 200 normal sera with Monobind Ferritin AccuLite™ CLIA procedure.

| Male’s | 15 - 230 ng/ml |
| Female’s | 10 - 126 ng/ml |

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. 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 analysts 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 precision of this method was determined by analyses on three different levels of control sera. The number mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

**TABLE 2**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>S</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>54.2</td>
<td>2.9</td>
<td>6.3%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>107.1</td>
<td>8.7</td>
<td>8.1%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>310.2</td>
<td>18.5</td>
<td>6.0%</td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>S</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>10</td>
<td>52.4</td>
<td>2.3</td>
<td>4.4%</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
<td>112.8</td>
<td>9.4</td>
<td>8.3%</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>301.5</td>
<td>21.2</td>
<td>7.0%</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

14.2 Sensitivity
The sensitivity detection limit) was ascertained by determining the variability of the 0mIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. It was determined to be 0.011 ng/ml.

14.3 Accuracy
The Ferritin AccuLite™ CLIA test was compared with a reference microparticle enzyme immunoassay (ELISA). Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 120. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

**TABLE 4**

<table>
<thead>
<tr>
<th>Method(Y)</th>
<th>Reference(X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method(Y)</td>
<td>Reference(X)</td>
</tr>
</tbody>
</table>

Only slight amounts of bias between the Monobind Ferritin CMA™ Microplate Procedure and the reference method are indicated by the closeness of the mean least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity
The cross-reactivity of the Ferritin AccuLite™ CLIA assay to other proteins was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of ferritin needed to produce the same light intensity.

15.0 REFERENCES

Revision: 3 Date: 061112 DCO: 0651 Cat #: 2875-300

For Orders and Inquiries, please contact Tel: 1401.991.2065 info@: info@monobind.com Fax: 965.991.5300 Web: www.monobind.com