K-ASSAY® Ferritin
For the Quantitative Determination of Ferritin Levels in Serum and Plasma
Cat. No. KAI-095

INTENDED USE

The K-ASSAY® Ferritin assay is an in vitro diagnostic reagent for the quantitative determination of ferritin (an iron-storing protein) in human serum and plasma by immunoturbidimetric assay on the Roche / Hitachi 917 and other analyzers. Measurements of ferritin aid in the diagnosis of diseases affecting iron overload and iron deficiency anemia. FOR IN VITRO DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

Ferritin is an iron-containing protein with a molecular weight of approximately 450,000 daltons. It is found mainly in the human liver and spleen, where its function is to eliminate and store iron, and is also found in small amounts in human serum. Ferritin is decreased in iron deficiency anemia and increased in iron overload. Ferritin levels correlate with and are useful in the evaluation of total body iron storage.

The K-ASSAY® Ferritin is a latex-enhanced immunoturbidimetric assay, developed to accurately and reproducibly measure ferritin levels in serum and plasma samples.

PRINCIPLE OF TEST

When an antigen-antibody reaction occurs between ferritin in a sample and an anti-ferritin antibody, which has been sensitized to latex particles, agglutination occurs. This agglutination is detected as an absorbance change, with the magnitude of the change being proportional to the quantity of ferritin in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known ferritin concentration.

KIT COMPOSITION

Reagents (Liquid stable)

R1: Buffer Reagent

1 x 18 mL

Glycine buffer solution (170 mM)

R2: Latex Suspension

1 x 9 mL

0.07% w/v suspension of latex particles sensitized with rabbit anti-human ferritin antibody.

REAGENT PREPARATION

Reagents are ready to use and do not require reconstitution.

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C) and protected from light. Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for 18 months from the date of manufacture as indicated on the expiration date on the package and bottle labels.

REAGENT STABILITY

Discard reagents if they become contaminated. Evidence of obvious precipitation in reagent 2 (R-2) solution is cause to discard. Opened reagents can be used for 1 month if stored at 2-8°C.

SPECIMEN COLLECTION AND PREPARATION

Serum

Blood should be collected from a patient and the serum separated as soon as possible. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot tube. For highly turbid samples, it is recommended that the sample be spun and the lipid layer be removed. It is recommended that the specimen collection be carried out in accordance with the NCCLS document H3-A2. After sampling, the specimen should be immediately stored at 2-8°C and assayed as soon as possible. If the assay cannot be performed within 24 hours, then the sample should be tightly capped and frozen at -20°C. Avoid repeated freeze-thaw cycles. Samples should be thawed at room temperature in a water bath or air. Undiluted samples should be used for this assay.

Plasma

Whole blood is collected in sodium EDTA or sodium heparin anticoagulant. After collection, immediately centrifuge. In the U.S., follow NCCLS guideline H3-A2. If the assay cannot be performed within 24 hours, then the sample should be tightly capped and frozen at -20°C. Avoid repeated freeze-thaw cycles. Samples should be thawed at room temperature in a water bath or air. Undiluted samples should be used for this assay.

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Solution

1 x 18 mL

Reagent 2 (R-2) Latex Suspension

1 x 9 mL

Materials Required But Not Supplied

Calibrators: K-ASSAY® Ferritin Calibrator, Cat. No. KAI-094C, 4 Calibrators: 100, 200, 500, 1,000 ng/mL.

Saline (0.9% w/v NaCl)

Roche / Hitachi 917 or other chemistry analyzer

Assay Procedure

An example of automated application:

Sample

7 µL

- R1 (Buffer Reagent) 140 µL

- R2 (Latex Suspension) 70 µL

Significance

Endpoint or Rate, 570 nm (main) / 800 nm (sub)

Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Roche / Hitachi 917</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>37°C</td>
</tr>
<tr>
<td>Test</td>
<td>FER</td>
</tr>
<tr>
<td>Assay Code</td>
<td>(2 POINT END) (10)</td>
</tr>
<tr>
<td>(18) (29)</td>
<td>(0) (0)</td>
</tr>
<tr>
<td>Wavelength</td>
<td>800 (780)</td>
</tr>
<tr>
<td>Sample Volume</td>
<td>7 (0) (0)</td>
</tr>
<tr>
<td>Reagent Vol (R1)</td>
<td>140 (0)</td>
</tr>
<tr>
<td>Reagent Vol (R2)</td>
<td>70 (0)</td>
</tr>
<tr>
<td>Reagent Vol (R3)</td>
<td>70 (0)</td>
</tr>
<tr>
<td>Reagent Vol (R4)</td>
<td>70 (0)</td>
</tr>
<tr>
<td>Abs Limit (Slope)</td>
<td>32000 (1 INCREASE)</td>
</tr>
<tr>
<td>Prozone Limit</td>
<td>32000 (0 LOWER)</td>
</tr>
<tr>
<td>Calib Type</td>
<td>SPLINE</td>
</tr>
<tr>
<td>Point</td>
<td>5</td>
</tr>
<tr>
<td>Span Point</td>
<td>5</td>
</tr>
<tr>
<td>SD Limit</td>
<td>999.9</td>
</tr>
<tr>
<td>Duplicate Limit</td>
<td>32000</td>
</tr>
<tr>
<td>Sensitivity Limit</td>
<td>0</td>
</tr>
<tr>
<td>Stabs Range</td>
<td>32000 (32000)</td>
</tr>
<tr>
<td>Instrument Factor</td>
<td>1 (1.0) (0.0)</td>
</tr>
<tr>
<td>Unit</td>
<td>ng/mL</td>
</tr>
<tr>
<td>STD (1) Conc.-POS.</td>
<td>1 (1)</td>
</tr>
<tr>
<td>STD (2) Conc.-POS.</td>
<td>1 (2)</td>
</tr>
<tr>
<td>STD (3) Conc.-POS.</td>
<td>1 (3)</td>
</tr>
<tr>
<td>STD (4) Conc.-POS.</td>
<td>1 (4)</td>
</tr>
<tr>
<td>STD (5) Conc.-POS.</td>
<td>1 (5)</td>
</tr>
<tr>
<td>STD (6) Conc.-POS.</td>
<td>1 (6)</td>
</tr>
</tbody>
</table>

Use isotonic saline as STD (1)

* 2-5 Input concentration of calibrators

Parameters for other automated analyzers are available.

CALIBRATION

A five-point calibration curve should be made using the K-ASSAY® Ferritin Calibrator and saline (0 ng/mL). In our laboratory, the calibration curve was stable for at least 4 weeks. It is recommended that the user determine calibration frequency on their analyzer as the calibration curve stability may change due to analyzer condition and use. Calibration is recommended whenever a new lot of reagent is used or when quality control material is outside the specified range.

QUALITY CONTROL

A quality control program is recommended for all clinical testing laboratories. It is recommended that controls, both normal and abnormal, be run with each batch of samples to monitor the procedure. Each laboratory should establish its own control range by assaying the control a sufficient number of times to generate a valid mean and acceptable range.

CALCULATIONS

Ferritin levels are determined using the prepared calibration curve.
EU AUTHORIZED REPRESENTATIVE

The measurable range for this ferritin test kit is between 2 ng/mL and 1,000 ng/mL. If the ferritin concentrations are greater than the highest calibrator value, dilute the sample with isotonic saline and re-assay. Multiply the result by the dilution factor to compensate for the dilution. Performance of this assay has only been evaluated on adult specimens. Since a reference range is only available for adult specimens, this assay should only be used for adults. Intra-fat concentrations greater than 3% have been shown to interfere with the assay, however, intra-lipid concentrations up to 5% do not interfere with the assay.

PERFORMANCE

Recovery

When a serum sample with a known ferritin value is assayed, the results obtained should be within ± 8%.

<table>
<thead>
<tr>
<th>Assigned Value</th>
<th>Measured</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>103</td>
<td>102.1</td>
</tr>
<tr>
<td>Sample 2</td>
<td>254</td>
<td>249.9</td>
</tr>
<tr>
<td>Sample 3</td>
<td>509</td>
<td>493.6</td>
</tr>
<tr>
<td>Sample 4</td>
<td>16</td>
<td>15.6</td>
</tr>
</tbody>
</table>

Precision

(Within Run)

Acceptance Criteria: When a sample is repeatedly assayed 10 times, the absorbance C.V. is less than 7%.

SERUM control samples were assayed 21 times on the same day.

Control I  Control II  Control III
N = 21  N = 21  N = 21
Mean = 14.9  Mean = 100.0  Mean = 431.1
Low = 13.8  Low = 98.7  Low = 427.6
High = 15.9  High = 101.2  High = 435.5
SD = 0.600  SD = 0.647  SD = 2.203
CV = 4.03%  CV = 0.65%  CV = 0.51%

(Between Runs)

Serum control samples were assayed on 20 different days.

Control I  Control II  Control III
N = 20  N = 20  N = 20
Mean = 16.5  Mean = 105.2  Mean = 428.7
Low = 15.4  Low = 102.7  Low = 421.0
High = 18.1  High = 107.6  High = 432.6
SD = 0.733  SD = 1.522  SD = 2.510
CV = 4.45%  CV = 1.45%  CV = 0.59%

Total Precision

<table>
<thead>
<tr>
<th>Control I</th>
<th>Total Precision = 3.44%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control II</td>
<td>Total Precision = 1.54%</td>
</tr>
<tr>
<td>Control III</td>
<td>Total Precision = 0.83%</td>
</tr>
</tbody>
</table>

Accuracy / Correlation

A comparison of the K-ASSAY® Ferritin and another company’s Ferritin on the Hitachi 917 was performed with the following results. Statistics were calculated by linear regression. Samples used in the correlation study were serum samples from Asian patients.

y = 0.895x + 4.037
r = 0.997
n = 64
x = Company A’s Ferritin
y = K-ASSAY® Ferritin
Slope 95% CI = ± 0.0177 (0.8723 to 0.9077)
Y-Intercept 95% CI = ± 6.443 (-15.8467 to -2.9608)

EXPECTED VALUES

226 normal male serum samples and 205 normal female serum samples were assayed for ferritin on a Roche/Hitachi 917 analyzer.

Male (n = 226) : The reference range was 7 - 253 ng/mL
Female (n = 205) : The reference range was 2 - 110 ng/mL

It is recommended that each laboratory establish its own expected range.

REFERENCES


LABELING SYMBOLS

- Lot Number
- Reagent
- Expiration or “Use By” Date
- Catalog Number
- For In Vitro Diagnostic Use
- Temperature Limitation.
- Manufacturer
- Consult Package Insert for Instructions for Use
- Authorized Representative in the European Community