ab108698

Ferritin Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Ferritin concentration in serum and plasma.

This product is for research use only and is not intended for \textit{in vitro} diagnostic use.

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1. Introduction

ab108698 Ferritin Human ELISA Kit is an immunoenzymatic colorimetric method for quantitative determination of Ferritin in human serum and plasma.

Ferritin is a globular protein found mainly in the liver, which can store about 2250 iron (Fe3⁺) ions. The ferritin molecule consists of a protein shell (apo-ferritin) composed of heavy and light subunits, which surrounds a crystalline core containing iron oxide and phosphate. Ferritin is synthesized in the liver, spleen and numerous other body tissues, with major concentrations found in the liver, spleen, bone marrow, and intestinal mucosa. The ferritin levels measured have a direct correlation with the total amount of iron stored in the body. If ferritin is high there is iron in excess, which would be excreted in the stool. If ferritin is low there is a risk for lack in iron, which sooner or later could lead to anaemia. In the setting of anaemia, serum ferritin is the most sensitive lab test for iron deficiency anaemia. In contrast, serum ferritin levels are normal or increased in anemia associated with chronic disease. Elevated serum ferritin levels have been observed in acute and chronic liver disease and lymphoid malignancy (leukemia and Hodgkin lymphoma). Ferritin is also used as a marker for iron overload disorders, such as haemochromatosis in which the ferritin level may be abnormally raised.
2. Assay Summary

*ab108698 is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system is based on simultaneous binding of human Ferritin to two monoclonal antibodies; one is immobilized on the microplate, the other is soluble and conjugated with horseradish peroxidase (HRP).*

Microtiter strip wells are precoated with anti-Ferritin IgG antibodies. Ferritin in samples and standards binds to the immobilised antibodies on the surface of the microtiter wells and the second, soluble anti- Ferritin antibody-enzyme conjugate binds to the immobile antibody- Ferritin -complex during the first incubation.

\[ \downarrow \]

Afterwards a bound/free separation is performed by solid-phase washing.

\[ \downarrow \]

The immune complex is visualized by adding Tetramethylbenzidine (TMB) substrate, which gives a blue reaction product. The intensity of this product is proportional to the amount of Ferritin in samples and standards.

\[ \downarrow \]

Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorption at 450 nm is read using an ELISA microwell plate reader.
3. Kit Contents

- **Anti-Ferritin IgG Coated Wells**: 12 break apart 8-well snap-off strips coated with anti-Ferritin IgG, in resealable aluminium foil.
- **Stop Solution**: 1 bottle containing 12 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **Anti-Ferritin-HRP conjugate**: 1 bottle containing 12 ml of horseradish peroxidase labeled anti Ferritin antibodies.
- **TMB Substrate Solution**: 1 bottle containing 12 ml 3, 3’, 5, 5’-tetramethylbenzidine (H₂O₂-TMB 0.25g/l) (avoid any skin contact).
- **Wash solution 20x conc.**: 1 bottle containing 50 ml (NaCl 9 g/l, Tween 20 1 g/l).
- **Ferritin Standards**: 6 bottles, 3 ml of standard 0, 1 ml each of all other standards. The standards are calibrated against the (WHO 1st IS Ferritin 80/602) and have the following concentrations:
  
<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:</td>
<td>5</td>
</tr>
<tr>
<td>2:</td>
<td>20</td>
</tr>
<tr>
<td>3:</td>
<td>100</td>
</tr>
<tr>
<td>4:</td>
<td>400</td>
</tr>
<tr>
<td>5:</td>
<td>1000</td>
</tr>
</tbody>
</table>

- **Strip holder**: 1
- **Cover foils**: 2
4. Storage and Handling

The reagents are stable up to the expiry date stated on the label when stored at 2 - 8 °C.

5. Additional Materials Required

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes of between 10 µl and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes
- Timer

6. Preparation of Reagents

*It is very important to bring all reagents, samples and standards to room temperature (20 - 25°C) before starting the test run.*

1. **Coated snap-off Strips:** The ready to use break apart snap-off strips are coated with anti-Ferritin IgG antibodies. Store at 2 - 8°C. *Immediately after removal of strips, the remaining strips*
should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 - 8°C. Do not remove the adhesive sheets on the unused strips.

2. **Anti-Ferritin-HRP conjugate:** The bottle contains 12 ml of a ready to use solution with anti-Ferritin antibodies conjugated with horseradish peroxidase.

3. **Ferritin Standards:** The standards are ready to use. *After first opening the standards are still stable for another 6 months at +4°C.*

4. **TMB Substrate Solution:** The bottle contains 12 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2-8°C in the dark. *The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.*

5. **Stop Solution:** The bottle contains a ready to use solution of 0.15 M sulphuric acid solution (R 36/38, S 26).

6. **Wash Solution:** Dilute the concentrated Wash Solution with distilled water to 1000 ml. Once diluted it is stable until the expiry date of the kit.
7. Preparation and Collection of Specimen

Use human serum or plasma (heparin, EDTA) samples with this assay. Specimen can be stored at 2 - 8°C for a short time (max five days). For longer storage the specimen should be aliquotted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

Samples with concentration of Ferritin over 1000 ng/ml have to be diluted with standard 0.

Precaution:

- The precoated microplate contains Proclin 300® as a preservative.
- Standards and conjugate contain Gentamycin as stabiliser.
- Do not use heavily haemolysed samples.
- Maximum precision is required for dispensation of the reagents.
- This method allows the determination of Ferritin from 5 ng/ml to 1000 ng/ml.
- Avoid exposure of TMB substrate to direct sunlight, metals or oxidants.
8. Assay Method

Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

<table>
<thead>
<tr>
<th>Wells</th>
<th>Example</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(e.g. A1)</td>
<td>for the substrate blank</td>
</tr>
<tr>
<td>2</td>
<td>(e.g. B1+C1)</td>
<td>for standard 0</td>
</tr>
<tr>
<td>2</td>
<td>(e.g. D1+E1)</td>
<td>for standard 1</td>
</tr>
<tr>
<td>2</td>
<td>(e.g. F1+G1)</td>
<td>for standard 2</td>
</tr>
<tr>
<td>2</td>
<td>(e.g. H1+A2)</td>
<td>for standard 3</td>
</tr>
<tr>
<td>2</td>
<td>(e.g. B2+C2)</td>
<td>for standard 4</td>
</tr>
<tr>
<td>2</td>
<td>(e.g. D2+E2)</td>
<td>for standard 5</td>
</tr>
</tbody>
</table>

*It is recommended to determine standards and samples in duplicate.*

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each sample.
Assay Procedure:

1. Dispense 20 µl standards and samples into their respective wells. Add 100 µl conjugate to each well. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour at room temperature (22 – 28°C).**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   
   *Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.*
5. Dispense 100 µl TMB Substrate Solution into all wells.
6. **Incubate for exactly 10 min at room temperature (22 – 28°C) in the dark.**
7. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
   
   *Any blue color developed during the incubation turns into yellow.*
8. Measure the absorbance of the specimen at 450 nm within 30 min after addition of the Stop Solution.
Measurement:

Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

*If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!*

Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard and sample.

Where applicable calculate the mean absorbance values of all duplicates.

Quality control:

Each laboratory should assay controls at normal, high and low levels range of Ferritin 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.

The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50
and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

9. Data Analysis

1. Calculate the mean absorbance for each point of the standard curve and each sample.
2. Plot the mean value of absorbance of the standards against concentration.
3. Draw the best-fit curve through the plotted points. (e.g. Cubic Spline or Four Parameter Logistic).
4. Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.
A. **Reference Values**

Each laboratory must establish its own normal ranges based on patient population.

The following values should be considered as a guideline:

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopausal females</td>
<td>6 – 180 ng/ml</td>
<td>53 ng/ml</td>
</tr>
<tr>
<td>Post-menopausal females</td>
<td>8 – 350 ng/ml</td>
<td>105 ng/ml</td>
</tr>
<tr>
<td>Males</td>
<td>20 – 400 ng/ml</td>
<td>175 ng/ml</td>
</tr>
</tbody>
</table>

B. **Sensitivity**

The lowest detectable concentration of Ferritin that can be distinguished from the standard 0 is 1.0 ng/ml at the 95 % confidence limit.

C. **Hook Effect**

ab108698 Ferritin Human ELISA Kit shows no Hook Effect up to 50,000 ng/ml.

D. **Recovery**

The recovery of 12.5 – 25 – 50 – 100 – 200 ng/ml of Ferritin added to sample gave an average value (±SD) of 98.4% ± 4.7%.
E. Reproducibility

**Intra-Assay:** Within-run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 4 %.

**Inter-Assay:** Between-run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 4.8 %.

10. Limitations

- Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.
11. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham:

<table>
<thead>
<tr>
<th>Material Tested</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver human Iso-Ferritin</td>
<td>100.0 %</td>
</tr>
<tr>
<td>Spleen human Iso-Ferritin</td>
<td>80.0 %</td>
</tr>
<tr>
<td>Heart human Iso-Ferritin</td>
<td>12.0 %</td>
</tr>
</tbody>
</table>
# 12. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td>Issue Description</td>
<td>Potential Cause</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
<td></td>
</tr>
<tr>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
<td></td>
</tr>
<tr>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
<td></td>
</tr>
<tr>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaw cycles)</td>
<td></td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wash wells as per protocol recommendations</td>
<td></td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
<td></td>
</tr>
<tr>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
<td></td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
<td></td>
</tr>
<tr>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
<td></td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
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