Ferritin

Enzyme immunoassay for the quantitative determination of Ferritin in human serum or plasma

Only for in-vitro diagnostic use

Product Number: DNOV100 (96 Determinations)
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1. INTRODUCTION

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 (apoferitin) 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 anaemia associated with chronic disease. Elevated serum ferritin levels have been observed in acute and chronic liver disease and lymphoid malignancy (leukemia and Hodgkin lymphoma). High serum ferritin levels have also been associated with an elevated risk for myocardial infarction in men. Ferritin is also used as a marker for iron overload disorders, such as haemochromatosis in which the ferritin level may be abnormally raised.

Ferritin is an acute-phase reactant, it is often elevated in the course of disease. Free iron is toxic to cells, and the body has an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored complexed to protein as ferritin or hemosiderin. Apoferritin binds to free ferrous iron and stores it in the ferric state. Under steady state conditions, the serum ferritin level correlates with total body iron stores; thus, the serum ferritin level is the most convenient laboratory test to estimate iron stores.

2. INTENDED USE

Immunoenzymatic colorimetric method (ELISA) for quantitative determination of Ferritin in serum or plasma.

3. PRINCIPLE OF THE ASSAY

The Ferritin assay 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 conjugated with HRP binds to the immobile antibody-Ferritin-complex during the first incubation. Afterwards a bound/free separation is performed by solid-phase washing. 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. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorption at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Anti-Ferritin IgG Coated Wells**: 12 breakapart 8-well snap-off strips coated with anti-Ferritin IgG; in resealable aluminium foil.
- **Stop Solution**: 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **Anti-Ferritin-HRP conjugate**: 1 bottle containing 12 ml of horseradish peroxidase labelled anti-Ferritin antibodies.
- **TMB Substrate Solution**: 1 bottle containing 15 ml 3, 3´, 5, 5´-tetramethylbenzidine (H₂O₂-TMB 0.26 g/l) (avoid any skin contact).
- **Wash Solution conc.**: 1 bottle containing 50 ml (NaCl 9 g/l and Tween-20 22 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:
  - Standard 0: 0 ng/ml
  - Standard 1: 5 ng/ml
  - Standard 2: 20 ng/ml
  - Standard 3: 100 ng/ml
  - Standard 4: 400 ng/ml
  - Standard 5: 1000 ng/ml

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan
4.3. Materials and Equipment needed
- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes
- Timer

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

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (22…28°C) before starting the test run!

6.1. 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; stability until expiry date. Do not remove the adhesive sheets on the unused strips.

6.2. Anti-Ferritin-HRP Conjugate
The bottle contains 12 ml of a ready to use solution with anti-Ferritin antibodies conjugated with horseradish peroxidase.

6.3. Standards
The standards are ready to use. After first opening standards are stable for another 6 months at 4 °C.

6.4. TMB Substrate Solution
The bottle contains 15 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 colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

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

6.6. Wash Solution
Dilute the concentrated wash solution to a volume of 1000 ml with distilled water in a suitable container. For smaller volumes respect the 1:20 ratio. The diluted wash solution is stable for 30 days at 2…8°C.

7. SPECIMEN COLLECTION AND PREPARATION
Use human serum or plasma 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 aliquoted and stored deep-frozen (-20°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.

7.1. Precaution
- The reagent contains Proclin 300® as a preservative.
- Standards and Conjugate contain gentamicin as stabiliser.
- Do not use heavily haemolysed samples.
- Maximum precision is required for dilution and dispensation of the reagents.
- This method allows the determination of Ferritin from 5 ng/ml to 1000 ng/ml.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.

8. ASSAY PROCEDURE

8.1. 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 on the result sheet supplied in the kit. 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 it lasts more than ten minutes, please follow the same order during the dispensation. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:
1 well (e.g. A1) for the substrate blank
2 wells (e.g. B1+C1) for standard 0
2 wells (e.g. D1+E1) for standard 1
2 wells (eg. F1+G1) for standard 2
2 wells (eg. H1+A2) for standard 3
2 wells (eg. B2+C2) for standard 4
2 wells (eg. D2+E2) for standard 5

It is recommended to determine standards and patient 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 patient sample.

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. Shake the microplate gently.

   Any blue colour 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.

8.2. 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 patient sample in the distribution and identification plan.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

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

9.2. Reference Values
The serum values are comprised in the following intervals:

<table>
<thead>
<tr>
<th>Category</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>

10. 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.
11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Precision

Intra Assay Variation
Within run variation was determined by replicate determination (16x) of three different control sera in one assay. The within assay variability is ≤ 5.4 %.

Inter Assay Variation
Between run variation was determined by replicate (16x) measurements of three different control sera in 2 different lots. The between assay variability is ≤ 6.1 %.

11.2. Specificity

The cross reaction of the antibody calculated on a weight/weight basis are:
- Liver human Iso-Ferritin 100.0 %
- Spleen human Iso-Ferritin 80.0 %
- Heart human Iso-Ferritin 12.0 %

11.3. Analytic Sensitivity

The lowest detectable concentration of Ferritin that can be distinguished from the standard 0 is 0.53 ng/ml at the 95 % confidence limit.

11.4. Accuracy

The recovery of 12.5 – 25 – 50 – 100 – 200 ng/ml of Ferritin added to sample gave an average value (±SD) of 98.66% ± 2.9%.

11.5. Correlation with RIA

The NovaTec Ferritin ELISA was compared to another commercially available Ferritin assay. Serum samples of 22 females and 32 males were analysed according in both test systems.

The linear regression curve was calculated

\[ \text{Ferritin NovaTec} = 1.11 \times \text{Ferritin Diasorin} - 10.46 \]

\[ r^2 = 0.972 \]

11.6. Hook Effect

The Ferritin ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 50,000 ng/ml.

12. LIMITATIONS OF THE PROCEDURE

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

13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
WARNING: In the used concentration Proclin 300 is hardly any toxicological risk upon contact with skin and mucous membranes!

WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

13.1. Disposal Considerations
Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

14. LITERATURE

15. ORDERING INFORMATION
Prod. No.: DNOV100 Ferritin Determination (96 Determinations)
SCHEME OF THE ASSAY  
Ferritin  

Test Preparation

Prepare reagents and samples as described.  
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.  
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

<table>
<thead>
<tr>
<th>Substrate blank</th>
<th>Standard 0</th>
<th>Standard 1</th>
<th>Standard 2</th>
<th>Standard 3</th>
<th>Standard 4</th>
<th>Standard 5</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 1</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
</tr>
<tr>
<td>Standard 5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
</tr>
<tr>
<td>Conjugate</td>
<td>-</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Cover wells with foil supplied in the kit.  
**Incubate for 1 h room temperature**  
Wash each well three times with 300 µl diluted Wash Solution.

<table>
<thead>
<tr>
<th>TMB Substrate</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
</tr>
</thead>
</table>

**Incubate for exactly 10 min at room temperature in the dark**

<table>
<thead>
<tr>
<th>Stop Solution</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
</tr>
</thead>
</table>

Shake the microplate gently.  
Photometric measurement at 450 nm

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