DRG® Ferritin ELISA (EIA-4408)

RUO in the USA

Revised 17 June 2009 (Vers. 2.0)

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of Ferritin concentration in serum and plasma

2 CLINICAL SIGNIFICANCE

Ferritin is a globular protein found mainly in the liver, which can store about 2'250 iron (Fe^{3+}) ions. The ferritin molecule consists of a protein shell (apoferritin) 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). 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.

3 PRINCIPLE

The Ferritin EIA TEST is based on simultaneous binding of human Ferritin to two monoclonal antibodies, one immobilized on microwell plates and the other conjugates with horseradish peroxidase (HPR).
After incubation the bound/free separation is performed by a simple solid-phase washing, then the TMB-Substrate solution (TMB) is added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbencies are determinated.
The Ferritin concentration in the sample is calculated based on a series of standard.
The colour intensity is proportional to the Ferritin concentration in the sample.

4 REAGENT, MATERIAL AND INSTRUMENTATION

4.1 Reagent and material supplied in the kit

1. **Zero Standard S0** (1 vial) 3 ml and
   Ferritin **Standards S1 – S5**, 5x (1 vial = 1 mL)

2. **Enzyme Conjugate** (1 bottle) 12 mL
   Anti Ferritin-HRP conjugate
3. **Coated Microplate** (1 microplate breakable)
   Anti-FerritinIgG adsorbed on microplate
4. TMB-**Substrate Solution** (1 bottle) 12 mL
   H_2O_2-TMB 0.25gr/L (avoid any skin contact)
5. **Stop Solution** (1 bottle) 12 mL
   Sulphuric acid 0.15 mol/L (avoid any skin contact)
6. Conc. **Wash Solution** (1 bottle) 50 ml
   NaCl 9 gr/L; Tween20 1gr/l

4.2 Reagents necessary not supplied

Distilled water.

4.3 Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader

Note
Store all reagents between 2°C – 8 °C in the dark.
Open the bag of reagent 3 (Coated Microplate) only when it is at room temperature and close immediately after use.
Do not remove the adhesive sheets on the strips unused.
Once open, the kit is stable up to expiration date.

5 PRECAUTION

- Reagent 3 contain Proclin 300 as preservative.
- The standards and reagent 2 contains gentamicin as stabilisers
- 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 5.0 to 1,000 ng/mL.
- To avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants.

6 PROCEDURE

6.1 Preparation of the Standard (S_0,S_1,S_2,S_3,S_4,S_5)

The standard calibrated against the (1st IS Ferritin WHO 80/602) has approximate the following concentration:

	S_0	S_1	S_2	S_3	S_4	S_5
ng/mL	0	5	20	100	400	1000

For sample with concentration over 1,000 ng/mL dilute the sample with S_0
Once open, the standards are stable 6 months at 4°C

Avoid direct contact with all potentially infected materials by using protective articles such as lab coats and disposable gloves.

6.2 Preparation of Wash Solution

Dilute contents of Concentrated Wash solution to 1L of distilled water.
Once diluted it is stable at 2°C – 8°C until the expire date of the kit.

6.3 Preparation of the Sample

Serum and plasma (heparin, EDTA) can be used.

No interferences of rheumatoid factor, bilirubin, haemoglobin, triglyceridis and cholesterol have been observed. Specimen can be stored at 2°C – 8°C for at short time (max five days). For longer storage the specimen should be frozen. Avoid repeated freezing and thawing.

6.4 Procedure

As it is necessary to perform the determination in duplicate, prepare two wells for each of the six points of the standard curve (S_0-S_5), two for each sample, one for Blank.
Pipette:

	Standard	Sample	Blank
Sample	---	20 µL	---
Standards S_0–S_5	20 µL	---	---
Conjugate	100 µL	100 µL	---

Incubate at room temperature for *1 hour*.
Remove the contents from each well and wash the wells with 300 µL of Diluted Wash Solution. Repeat the washing procedure two times by draining the wash completely.

Pipette

	Standard	Sample	Blank
TMB-Substrate	100 µL	100 µL	100 µL

Incubate at room temperature 22÷28°C for *10 minutes* in the dark.

Pipette:

	Standard	Sample	Blank
Stop Solution	100 µL	100 µL	100 µL

Read the absorbance (E) at 450 nm against Blank.

7 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.

8 LIMITATION OF PROCEDURE

8.1 Assay Performance

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemeic or haemolysed specimen(s) should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. 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. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

8.2 Interpretation of results

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 RESULTS

Mean Absorbance

Calculate the mean of the absorbencies (Em) corresponding to the single points to the standard curve (S0-S5) and of each sample.

Standard Curve

Plot the values of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (es:Cubic Spline or Four Parameter Logistic).

Calculation of Results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

10 REFERENCE VALUE

The serum or plasma values are comprised in the following intervals:

	Mean (ng/mL)	Range (ng/mL)
Females:		
Premenopausal	53	6 - 180
Post-menopausal	105	8 – 350
Males	175	20 - 400

11 PERFORMANCE AND CHARACTERISTICS

11.1 Precision

Intra Assay Variation

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 Variation

Between run variations was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 4.8%.

11.2 Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Liver Human Iso-Ferritin	100.0 %
Spleen Human Iso-Ferritin	80.0 %
Hearth Human Isoferritin	12.0 %

11.3 Accuracy

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%.

11.4 Sensitivity

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

11.5 Correlation with RIA

The 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

$y = 1.10 x – 10.4$

$r = 0.99$ ($r^2 = 0.98$)

11.6 Hook Effect

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

12 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

13 BIBLIOGRAPHY

Walter G.O., et al J. Clin. Path . 29 770 - 772 (1973)
Watanabe, N. et al Clin. Chem.,25/1 , 80 – 82 (1979)
Van Oost, S.A. et al Clin. Chem ,28/12 ,2429 – 2433 (1982)
Ronald H, et al Clin. Chem 29/6, 1109 - 1113 (1983)

14 TROUBLESHOOTING

ERRORS / POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed)

too high within-run CV%

- reagents and/or strips not pre-warmed to room temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run CV %

- incubation conditions not constant (time, temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

DRG

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CE

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Revised 17 June 2009 (Vers. 2.0)

SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..