Ferritin IRMA

KIPB3492

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History

Summary of change :

Previous Version : 200224/1	**Current Version :** 240412
Old DiaSource logo	New DiaSource logo on the front page
Contact address tech.support@diasource.be	Correction of the contact address products.support@diasource.be

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Ferritin IRMA

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Immunoradiometric assay for the in vitro determination of ferritin in human serum and plasma

KIPB3492

IN VITRO DIAGNOSTIC

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1. INTENDED USE

The measurement of ferritin in serum and plasma is useful in evaluating iron levels in the body.

In oncology, the determination of Ferritin is used in the management of patients with acute myeloblastic leukemia, malignant lymphomas, renal cell carcinoma and to detect recurrent disease in follow up care.

2. PRINCIPLE OF THE ASSAY

The immunoradiometric assay of ferritin is a "sandwich" type assay. Mouse monoclonal antibodies directed against two different epitopes of ferritin molecule and hence not competing are used. The samples or calibrators are incubated in tubes coated with the first monoclonal antibody in the presence of the second monoclonal antibody labeled with iodine 125. After incubation, the content of tubes is aspirated and the tubes are rinsed so as to remove unbound ^{125}I-labeled antibody. The bound radioactivity is then determined in a gamma counter. The ferritin concentrations in the samples are obtained by interpolation from the calibration curve. The concentration of ferritin in the samples is directly proportional to the radioactivity.

3. REAGENTS PROVIDED

All reagents of the kit are stable until the expiry date indicated on the kit label, if stored at 2-8°C. Expiry dates printed on vial labels apply to the long-term storage of components by the manufacturer only, prior to assembly of the kit. Do not take into account.
Storage conditions for reagents after dilution are indicated in paragraph Assay Procedure.

Anti-ferritin monoclonal antibody-coated tubes:
2 x 50 tubes (ready-to-use)

Ab	125I

^{125}I-labeled monoclonal anti-ferritin antibody: 1 x 55 mL vial (ready-to-use)
The vial contains 325 kBq, at the date of manufacture, of ^{125}I-labeled immunoglobulins in buffer with Proclin 300 (<0.06%; See precautions) bovine serum albumin and a dye.

CAL	N

Calibrators: 5 x 0.5 mL vials and 1 x 5 mL vial of «zero» calibrator (ready-to-use)
The calibrator vials contain from 0 to approximatively 1200 ng/mL of ferritin in buffer with Proclin 300 (<0.06%; See Precautions) bovine serum albumin. The exact concentration is indicated on each vial label. The calibrators were calibrated using the international standard 3rd IS NIBSC (recombinant ferritin) 94/572.

CONTROL	N

Control sera: 2 x 0.5 mL vials (ready-to-use)
The vials contain ferritin with Proclin 300 (<0.06%) in human serum (see Precautions).The expected values are in the concentration range indicated on the vial label.

WASH	SOLN	CONC

Wash solution (20x): 1 x 50 mL vial
Concentrated solution has to be diluted before use.

4. MATERIAL REQUIRED BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:
- Precision micropipets (20 µL).
- Semi-automatic pipets (500 µL, 2 mL).
- Vortex type mixer.
- Horizontal or orbital shaker.
- Aspiration system.
- Gamma counter set for 125 iodine.

5. PRECAUTIONS

5.1 General remarks:
- The vials with calibrators and controls should be opened as shortly as possible to avoid excessive evaporation.
- Do not mix the reagents from kits of different lots.
- A calibration curve must be established with each assay.
- It is recommended to perform the assay in duplicate.
- Each tube must be used only once.

5.2 Basic rules of radiation safety
The purchase, possession, utilization, and transfer of radioactive material are subject to the regulations of the country of use.
Adherence to the basic rules of radiation safety should provide adequate protection:
- No eating, drinking, smoking or application of cosmetics should be carried out in the presence of radioactive materials.
- No pipeting of radioactive solutions by mouth.
- Avoid all contact with radioactive materials by using gloves and laboratory overalls.
- All manipulation of radioactive substances should be done in an appropriate place, distant from corridors and other busy places.
- Radioactive materials should be stored in the container provided in a designated area.
- A record of receipt and storage of all radioactive products should be kept up to date.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes.
- Each case of radioactive contamination or loss of radioactive material should be resolved according to established procedures.
- Radioactive waste should be handled according to the rules established in the country of use.
- This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations

5.3 Material of human origin
The materials of human origin, contained in this kit, were found negative for the presence of antibodies to HIV 1 and HIV 2, antibodies to HCV, as well as of Hepatitis B surface antigen (HBsAg). However, they should be handled as if capable of transmitting disease. No known test method can offer total assurance that no virus is present. Handle this kit with all necessary precautions.
All serum and plasma samples should be handled as if capable of transmitting hepatitis or AIDS and waste should be discarded according to the country rules.

5.4 ProClin 300

R43 may cause sensitisation by skin contact

5.5 GHS hazard classification
Tracer WARNING
H317 May cause an allergic skin reaction.
P261 Avoid breathing vapours.
P272 Contaminated work clothing should not be allowed out of the workplace.
P280 Wear protective gloves, protective clothing and eye/face protection.
P302+P352 IF ON SKIN: Wash with plenty of soap and water.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before use.
P501 Dispose of contents/container in accordance with local/national regulations reaction mass of:
5-chloro-2-methyl-4-isothiazolin-3-one[EC#247-500-7]and2-methyl-4isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%

Control / Calibrator WARNING
H317 May cause an allergic skin reaction.
P261 Avoid breathing vapours.

P272 Contaminatedworkclothing should not be allowed out of the workplace.
P280 Wear protective gloves, protective clothing and eye/face protection.
P302+P352 IF ON SKIN: Wash with plenty of soap and water.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before use.
P501 Dispose of contents/container in accordance with local/national regulations
reaction mass of:
5-chloro-2-methyl-4-isothiazolin-3-one[EC#247-500-7]and2-methyl-4isothiazolin-3-one [EC# 220-239-6](3:1) <0.05%

Wash solution (20x) DANGER
H360 May damage fertility or the unborn child.
P201 Obtain special instructions before use.
P280 Wear protective gloves, protective clothing and eye/face protection.
P308+P313 IF exposed or concerned: Get medical advice/attention.

6. SPECIMEN COLLECTION, PROCESSING, STORAGE AND DILUTION

- Collect blood in tubes containing no additives or EDTA
- Separate serum or plasma from cells by centrifugation.
- Serum and plasma samples may be stored at 2-8°C, if the assay is to be performed within 24 hours. For longer storage keep frozen (at <-20°C), for up to 1 year, after aliquoting so as to avoid repeated freezing and thawing.
  Thawing of samples should be performed at room temperature.
- If samples have concentrations greater than the highest calibrator they must be diluted by zero calibrator.

Serum and EDTA plasma values for 15 samples (serum values ranging from 7.77 to 206.04 ng/ml) were compared using the KIPB3492 Ferritin IRMA kit.
Results are as follows:
[EDTA-plasma]=0.9236(serum) + 4.1765
R=0.9958

7. ASSAY PROCEDURE

7.1 Preparation of reagents

Let all the reagents come to room temperature.

7.1.1 Preparation of the wash solution

Pour the content of the vial into 950 mL of distilled water and homogenize.
The diluted solution may be stored at 2-8°C until the expiry date of the kit.

7.2 Assay procedure:

Step 1 Additions	Step 2 Incubation	Step 3 Counting
To coated tubes, add successively: - 20 µL of calibrator, control or sample and - 500 µL of tracer. Mix.	Incubate 1 hour at 18-25°C with shaking (>280 rpm).	Aspirate carefully the contents of tubes (except the 2 tubes «total cpm»). Wash with 2 mL of wash solution and aspirate twice. Count bound cpm (B) and total cpm (T) for 1 min.

* Add 500 µL of tracer to 2 additional tubes to obtain total cpm.

8. RESULTS

Results are obtained from the calibration curve by interpolation. The curve serves for the determination of ferritin concentrations in samples measured at the same time as the calibrators.

8.1 Calibration curve

The results in the package insert were calculated using a log-log curve fit ("spline" mode) with determined radioactivity ($cpm_{cal} - cpm_{cal0}$) on vertical axis and the ferritin concentration of the calibrators on the horizontal axis (ng/mL). Other data reduction methods may give slightly different results.

Total activity: 107.924 cpm

Calibrators	Ferritin (ng/mL)	cpm (n=3)	B/T (%)	$cpm_{cal} - cpm_{cal0}$
0	0	59	0.05	-
1	4.5	529	0.49	470
2	17.0	2,040	1.89	1.981
3	85.0	9.392	8.70	9.333
4	455	44,022	40.8	43,963
5	1100	70,607	65.4	70,548

(Example of calibration curve, do not use for calculation)

8.2 Samples

Locate for each sample the ($cpm_{sample} - cpm_{cal0}$) value on the vertical axis and read off the corresponding ferritin concentration of the sample on the horizontal axis in ng/mL. The concentrations of diluted samples must be corrected by the dilution factor.

9. QUALITY CONTROL

Good laboratory practices imply that control samples must be used regularly to ensure the quality of the results obtained. These samples must be processed exactly in the same way as the assay samples, and it is recommended to analyse their results using appropriate statistical methods.

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address:

products.support@diasource.be

10. EXPECTED VALUES

It is suggested that each laboratory establishes its own normal values. The following values obtained with 693 healthy subjects are indicative only.

	N	Conc. range at 95% confidence level	Median
Men	343	38 to 457 ng/mL	128 ng/mL
Pre-menopausal women	211	7.4 to 73 ng/mL	23.1 ng/mL
Menopausal women	139	14 to 165 ng/mL	62.1 ng/mL

Children

The following values obtained with 426 healthy children are indicative only

	N	Conc. range at 95% confidence level	Median
Children 0-6 months	45	1.0 – 434 ng/mL	91 ng/mL
Children 0.5 – 15 years	340	2.0 – 135 ng/mL	17.5 ng/mL
boys 15-18 years	28	16 – 90 ng/mL	36 ng/mL
girls 15 -18 years	13	5.0 – 127 ng/mL	15 ng/mL

Ferritin concentrations are age-dependent. They are also influenced by the lack of iron caused by menstrual bleeding in women. Therefore we recommend that each laboratory establish its own reference values, based on clinically characterized data.

11. PERFORMANCE CHARACTERISTICS

(For more details, see the data sheet "APPENDIX")

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary

11.1 Sensitivity

11.1.1 Analytical sensitivity: 0.39 ng/mL

11.1.2 Functional sensitivity: 3.2 ng/mL

11.2 Specificity

Cross-reaction of ferritin from various tissues:
spleen ferritin ..... 100 %
liver ferritin ..... 35 %
heart ferritin ..... 2.2 %
placental ferritin ..... 227 %

11.3 Precision

11.3.1 Intra-assay

Serum samples were assayed in 25 times in the same series. The coefficients of variation were found below or equal to 6.3 %.

11.3.2 Inter-assay

Serum samples were assayed in duplicate in 10 different series. The coefficients of variation were found below or equal to 5.8 %.

11.4 Accuracy

11.4.1 Dilution test

High-concentration serum samples were serially diluted with zero calibrator.
The recovery percentages obtained were between 89.6 % and 120 %.

11.4.2 Recovery test

Equal volumes of two ferritin serum samples with known ferritin concentrations were mixed and assayed according to the assay procedure. The recovery percentages obtained were between 89.3 % and 115 %.

11.5 Measurement range (from analytical sensitivity to highest calibrator):
0.39 to approximatively 1200 ng/mL.

12. LIMITATIONS OF THE PROCEDURE

The non-respect of the instructions in this package insert may affect results significantly. Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other appropriate information.
Do not use hemolyzed, lipemic or icteric samples.

"Hook effect", using this ferritin IRMA kit, does not appear in samples containing ferritin concentrations equal to or lower than 25,000 ng/mL. It is recommended that samples suspected of containing very high concentrations of ferritin are diluted with the zero calibrator.

For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays.
Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.