RK-900CTACE160401

FERRITIN [I-125] IRMA KIT

(REF: RK-900CT)

The FERRITIN[^{125}I] IRMA system provides a direct quantitative determination of FERRITIN in human serum. FERRITIN can be assayed in the range of 0-1000 ng/ml. Each kit contains materials sufficient for 100 assay tubes permitting the construction of one standard curve and the assay of 42 unknowns in duplicate.

Introduction

Ferritin is the main storage protein for iron (Mw:450000) containing protein shell and a crystalline core iron oxide and phosphate. Low serum ferritin level is a useful diagnostic marker for iron-deficiency anemia. High ferritin levels may indicate iron overload in the case of hemochromatosis. Elevated serum ferritin levels may also be observed in acute and chronic liver disease.

Principle of the method

The technology uses two high affinity monoclonal antibodies in an immuno-radiometric assay (IRMA) system. The ^{125}I labelled signal-antibody binds to an epitope of the ferritin molecule, which is different from that recognised by the unlabelled capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During a 1-hour incubation period with continuous agitation immuno-complex is immobilized on the reactive surface of test tubes. Reaction mixture is then discarded, test tubes are washed exhaustively, and Label coated tubes in duplicate for each the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of standards containing known amount of ferritin, the unknown concentration of ferritin in patient samples can be determined.

Contents of the kit

1. 1 bottle of TRACER, 21 ml, ready to use. Contains less than 740 kBq of ^{125}I labelled anti-FERRITIN and biotin labelled anti-FERRITIN in buffer containing proteins, 0.1% sodium azide, red coloured.

2. 6 vials of STANDARDS (S1-S6), ready to use (S1) 2.5 ml, (S2-S6) 0.5 ml equine serum containing spleen ferritin and 0.1% NaN_3.

Conc.*: 0, 5, 20, 70, 270, 1000 ng/ml.*

3. 1 vial of CONTROL SERUM, ready to use. 0.5 ml human serum, containing 0.1% NaN_3.

The concentration of control serum is specified in the quality certificate enclosed.

4.. 2 boxes of COATED TUBES, ready to use. 2x50 plastic tube, coated with streptavidin**.**

5. 1 bottle of WASH BUFFER CONCENTRATE, 20 ml, with 0.2% NaN_3. Dilute with 700 ml distilled water before use.

Quality certificate

Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips for 40 µl, repeating pipettes for 200 and 2000 µl, shaker, plastic foil, adsorbent tissue, gamma counter

Recommended tools and equipment

Dispenser with reservoir (instead of the 2-ml pipette)

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Do not use lipemic, hemolyzed or turbid specimens. Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided.

Preparation of reagents, storage

Store the reagents between 2-8°C after opening. At this temperature reagents are stable until expiry date. The actual expiry date is given on the package label and in the quality certificate.

Add the *wash buffer concentrate* to 700 ml distilled water. The diluted solution can be stored at 2-8°C until expiry date of the kit.

CAUTION! Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide, refer to Table 1.)

1. standard (S1-S6), control serum(C) and samples(P). Optionally, label two test tubes for total count (T).
2. Pipette **40 µl** each of STANDARD (S1-S6), CONTROL(C) and SAMPLES (P) into the properly labeled tubes.
3. Pipette **200 µl** of TRACER into each tube.
4. Seal all tubes with a plastic foil. If optional total counts tubes are also prepared, place them separately from others.
5. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube. Incubate tubes for 1 hour at room temperature. *(Note: The efficient rotation is a critical factor to achieve good performance. An uneven or incomplete shaking may result in a serious error. Minimum 600 rpm recommended)*
6. Add 2 ml diluted wash buffer to each tube and decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
7. Repeat Step 6.
8. Count each tube for at least 60 seconds in a gamma counter.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

	T	S1-S6	C	P
Standard		**40**		
Control			**40**	
Samples				**40**
Tracer	**200**	**200**	**200**	**200**
Vortex mix Rotate for 1 hour at room temperature				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

Calculate the average CPM for each pair of assay tubes. Draw the standard curve by plotting mean CPM of each standard level (ordinate) against the respective concentration, except for 0 standard (abscissa) using log-log graph paper.

Obtain sample concentration by interpolation of sample counts on the standard curve.

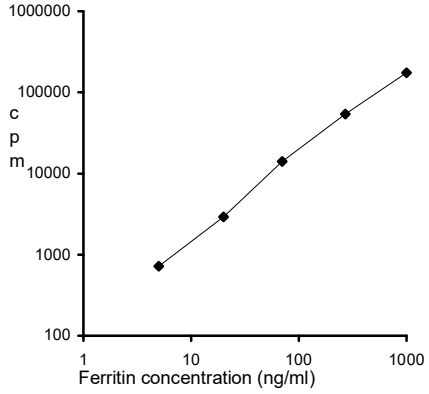
For computerized calculations and/or quality assessment normalized specific binding values, rather than cpm values are used. Specific binding values can be calculated for each standard and sample according to the following equation:

$$B/T\ (\%) = \frac{\text{S2-6/C/P (cpm)} - \text{S1(cpm)}}{\text{T (cpm)}} \times 100$$

Table 2. Typical assay data

Tubes	Mean cpm	B/T%	ng/ml
T	261609		
S1	116	0.04	
S2	924	0.35	
S3	3241	1.24	
S4	11032	4.22	
S5	45032	17.21	
S6	129971	49.68	
C	12755		78.4

A typical standard curve



Characterization of the assay

Performance parameters have been determined under ideal experimental conditions; by using fresh tracers.

Calibration
Standards are calibrated against the WHO International Standard, Code 94/572

Typical assay parameters
NSB/T < 0.06%
B_{max}/T > 40 %

Analytical sensitivity
The analytical sensitivity of this assay is 0.6 ng/ml calculated from the 2 x SD value at zero standard and from the slope of the curve at zero dose.

Hook effect
There is no high dose "hook effect" up to a FERRITIN concentration of 74000 ng/ml.

Specificity
Cross-reactivity for human liver ferritin is 100 %.

Precision and reproducibility
5 samples with 20 replicates in 1 assay run, and 7 samples with duplicates in 12 runs were measured to determine intra-assay and inter-assay precision, respectively. Values obtained are shown below:

Intra-assay		Inter-assay	
Mean (ng/ml)	CV %	Mean (ng/ml)	CV %
20.3	1.95	2.19	7.65
38.5	2.90	40.2	2.76
90.2	1.45	67.3	5.12
365	1.21	92.4	4.43
448	2.49	195.5	3.83
		366.4	3.76
		506.8	3.67

Recovery
Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of ferritin. Values for 11 serum samples spiked with FERRITIN (91 ng/ml) were as follows: 92.9 ± 8.7 %

Dilution test
6 samples were measured in a series of dilution (3, 9, 27-fold) with zero-standard. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:
y = 0.934x + 0.83, R = 0.9999, n=18

Expected values
It is recommended that each laboratory establish its own reference intervals. The expected values presented here are based on testing of apparently healthy blood donors. Samples were measured in duplicates.
Serum ferritin results were normally distributed after log transformation. Normal range is calculated on the basis of mean ± 2SD (log scale).
In a population (n=98) of **adult female** blood donors (ages: mean 32.9 ± 9.5, range 19 - 49) serum concentrations of ferritin were 33,8 ± 27.4 ng/ml (mean ± SD). Sample values were found scattered in a range of 8.4-170 ng/ml.

Reference range: 6-111 ng/ml

In a population (n=55) of **adult female** blood donors (ages: mean 55.6 ± 3.8, range 50-66) serum concentrations of ferritin were 47.7 ± 46.7 ng/ml (mean ± SD). Sample values were found scattered in a range of 6.7-224 ng/ml.

Reference range: 7 - 172 ng/ml

In a population (n=99) of **adult male** blood donors (ages: mean 36.7 ± 13 range 19 - 64) serum concentrations of ferritin were 102.2 ± 100.7 ng/ml (mean ± SD). Sample values were found scattered in a range of 10-299 ng/ml.

Reference range: 17 - 321 ng/ml

Conversion of values
1 nmol/l = 450 ng/ml
1 ng/ml = 2.22 pmol/l

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

3) **Addition of wash buffer.** For the addition of wash buffer the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precautions and warnings

Radioactivity
This kit contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard
Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1/2) Hepatitis B surface Antigen (HBsAg), Hepatitis C antibody and Treponema antibody. Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.
All animal products and derivatives have been collected from healthy animals.

Chemical hazard
Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 66.5 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 60 days from availability.

	Use-by date	LOT	Batch code
2 8	Temperature limitation Store between 2-8 °C	CONTROL	Control
	Caution, consult accompanying documents	CAL	Standard
	Biological risks	CT	Coated Tube
	Consult instructions for use	TRAC	Tracer
IVD	*In vitro* diagnostic medical device	WASHB	Wash Buffer
	Manufacturer		Radioactive material
REF	Catalogue number	CE	

WEB site: http://www.izotop.hu
Technical e-mail: immuno@izotop.hu
Commercial e-mail: commerce@izotop.hu

IZOTOP
INSTITUTE OF ISOTOPES Ltd.
1535 Budapest. Pf.: 851.
Tel.: (+36)1-392-2577, Fax: (+36)1-395-9247

Updated: April/2016