Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

Ferritin Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAFER1	Ferritin Elisa	96 Tests

Intended Use:

Ferritin Elisa is intended to be used for the quantitative determination of Ferritin in Human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

Iron storage compounds in the body include haemoglobin, haemosiderin, myoglobin and the cytochromes. In most tissues, Ferritin is a major iron-storage protein. Human Ferritin has a molecular weight of 450 Kd and consists of a protein shell around an iron core; each molecule of Ferritin may contain as many as 4000 iron atoms. Under normal conditions this may represent 25% of the total iron found in the body. In addition, Ferritin can be found in several isomers. High concentrations of Ferritin are found in the cytoplasm of the reticuloendothelial system, the liver, spleen and bone marrow. High Ferritin concentrations may indicate Iron overload without apparent liver damage, as may be noted in early stages of idiopathic hemochromatosis. Ferritin levels in serum have also been used to evaluate clinical conditions not related to iron storage including inflammation chronic liver disease and malignancy.

The Ferritin Elisa is based on a solid phase EIA. The assay system utilizes one anti-FERRITIN antibody for solid phase immobilization and another mouse monoclonal anti-FERRITIN antibody in the antibody enzyme conjugate solution. The test sample is allowed to react simultaneously with the antibodies resulting in the FERRITIN molecules being sandwiched between the solid phase and enzyme linked antibodies. After 60 minute incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes resulting in the development of a blue colour. The colour development is stopped with the addition of 2N HCl, and the colour is changed to yellow and measured at 450nm. The concentration of FERRITIN is directly proportional to the intensity of colour developed.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with monoclonal anti FERRITIN antibodies. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 2 months at 2-8°C.
FERRITIN Calibrators	6x1ml	6 vials containing FERRITIN at concentrations of 0.0, 10, 50, 100, 400 and 800 ng/ml made up in a human serum matrix. Reference against NIBSC WHO 80/602 THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable for 1 month at 2-8°C.
Enzymatic Conjugate	1x12ml	1 vial containing 12ml of HRP labelled monoclonal Anti FERRITIN antibodies in Buffered saline. Once open, stable for 2 months at 2-8°C.
Wash Buffer Concentrate (50X)	1x15ml	PBS-Tween at pH 7.4. 50X concentrate. The concentrate must be diluted with 735ml of distilled water before use. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x12ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable for 2 months at 2-8°C.
Stop Solution	1x12ml	Diluted Sulfuric acid solution (1M) Ready to use. Once open, stable for 2 months at 2-8°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

Materials required but not provided:

Distilled water, Vortex mixer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection:

Serum should be prepared from whole blood specimen obtained by acceptable medical techniques. The kit is for use with serum samples without additives only.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag provided and return to 2-8°C, under which conditions the wells will remain stable for 2 months, or until the labelled expiry date, whichever is earlier. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labelled expiry date, whichever is earlier.

Procedure:

Reagent preparation:

1. Bring all reagents to room temperature (18-22°C) prior to use.
2. Dilute the wash buffer concentrate with 735ml of Distilled water (yielding a total volume of 750ml). Once diluted the wash solution is stable for 2 months at room temperature. Mix well before use.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series.

STEP 2

Addition of Samples and calibrators and Enzyme Conjugate: Add 20ul of Calibrators and Samples to each well and 100ul of the Enzyme conjugate reagent into each well. Mix thoroughly for 30 seconds. Mixing is a very important step.

STEP 3

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at room temperature (18-22°C).

STEP 4

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer of 350ul. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 5

Addition of the Substrate: Add 100ul of Substrate Solution to each well. Mix gently for 5 seconds.

STEP 6

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 7

Stopping the Reaction: Add 100ul of the Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to yellow from blue.

STEP 8

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader within 15 minutes of adding the Stop Solution. Note down the absorbances.

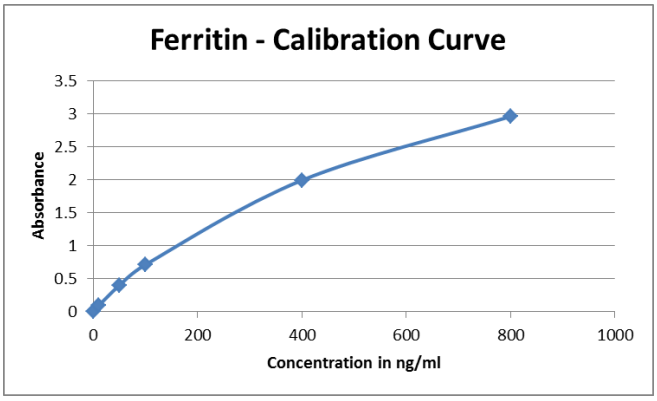
Note: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in ng/ml in X axis.
- Draw a point to point curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	0.003	0.0 ng/ml
CAL B	0.093	10.0 ng/ml
CAL C	0.401	50.0 ng/ml
CAL D	0.714	100.0 ng/ml
CAL E	1.995	400.0 ng/ml
CAL F	2.963	800.0 ng/ml



The above calibration curve is for illustrative purposes only and should not be used in lieu of an actual calibration.

Expected Values:

Each laboratory must establish its own normal ranges based on patient population. The results provided below are based on a limited number of healthy adult blood specimens.

When assayed with 80 Males and 90 females the mean in ng/ml for Ferritin was detected as 170 ng/ml for males and 71 ng/ml for females.
The ranges for the normal reference values can be used as 32.0 to 501 ng/ml for males and 3.5 – 223.5 for females.

Performance Characteristics:

1. **Intra assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	20	326.28 ng/ml	15.20	4.66 %
2	20	189.08 ng/ml	10.41	5.51 %
3	20	26.52 ng/ml	1.72	6.49 %

2. **Inter assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	20	332.83 ng/ml	20.46	6.15 %
2	20	192.18 ng/ml	14.33	7.46 %
3	20	24.24 ng/ml	3.58	14.73%

3. **Sensitivity:**

The minimum detectable concentration of FERRITIN by this assay was found to be 5 ng/ml.

Linearity:

A patient serum was serially diluted with 0 ng/ml standard in a linearity study. The average recovery was 102.2%

Sample A			
Dilution	Expected	Observed	% Recovery
Sample undil	186.94 ng/ml	186.94 ng/ml	
2x	93.47 ng/ml	94.16 ng/ml	100.7 %
4x	46.74 ng/ml	48.76 ng/ml	104.3 %
8x	23.37 ng/ml	24.53 ng/lm	105.0 %
16x	11.69 ng/ml	12.34 ng/ml	105.6 %
32x	5.85 ng/ml	5.87 ng/ml	100.3 %
Average Recovery: 103.2%			

Sample B			
Dilution	Expected	Observed	% Recovery
Sample undil	146.25 ng/ml	146.25 ng/ml	
2x	73.13 ng/ml	76.51 ng/ml	104.6 %
4x	36.57 ng/ml	37.50 ng/ml	102.5 %
8x	18.29 ng/ml	18.01 ng/lm	98.5 %
16x	9.15 ng/ml	8.73 ng/ml	95.5 %
32x	4.58 ng/ml	4.27 ng/ml	93.2 %
Average Recovery: 103.2%			

Method Comparison:

Method comparison between this assay and a commercially available assay yielded the following data:
N=115, Correlation Coeff: 0.990, Slope: 0.817, Intercept: -3.74
Mean values: This assay: 195.1 ng/ml and comparator: 243.4 ng/ml

Recovery:

Various patient serum samples of FERRITIN levels were mixed and assayed in duplicate. The average recovery was 98.1%

Expected conc ng/ml	Observed Conc ng/ml	% Recovery
5.07	5.05	99.6 %
9.30	8.79	94.5 %
21.27	20.11	94.5 %
43.13	48.03	89.7 %
85.34	90.00	105.5 %
166.60	174.06	104.4 %
Average Recovery: 98.1%		

Cross Reactivity:

The following materials were tested for cross reactivity and the results are as follows:

Antigens	Concentration	Equivalent FERRITIN
HS Albumin	10 g/dl	0.0 ng/ml
Human AFP	8000 ng/ml	0.0 ng/ml
Ferric Chloride	100 mg/dl	0.0 ng/ml
Human Transferrin	100 mg/dl	0.0 ng/ml
Human Haemoglobin	500 mg/dl	0.0 ng/ml

No High dose hook effect was observed up to 1200 ng/ml of ferritin in this assay.

References:

1. White D; Kramer D; Johnson, G, Dick, F and Hamilton H. Am J Cl 72: 346; 1986
2. Valberg, L. CMAJ. 122: 1240; 1980
3. Forman D and Parkar, S. Ann Clin Lab Sci 10: 345; 1980.
4. Hazard, J.T.: Yokota, M; Arosio,P and Drysdale, J. Blood. 49: 139; 1977.
5. Smimes, M.A,: Addiego, Jr. J.E.and Dallman, P.R. Blood. 43: 581; 1974

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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