Ferritin ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIAFER1	Ferritin ELISA	96 Tests

Intended Use:

The Ferritin ELISA is intended to be used for the quantitative determination of Ferritin in human serum. This kit is for *in vitro* diagnostic use only.

Summary and Principle:

Iron storage compounds in the body include haemoglobin, haemosiderin, ferritin, myoglobin and the cytochromes. In most tissues, Ferritin is the 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 up to 4000 iron ions. 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 operates under the sandwich ELISA principle. The assay system uses one anti- ferritin antibody immobilized on the solid phase and a mouse monoclonal anti- ferritin antibody in the enzyme conjugate reagent. The test sample is allowed to react simultaneously with the antibodies resulting in ferritin molecules present in the sample being sandwiched between the solid phase and enzyme linked antibodies. After incubation, the wells are washed to remove unbound sample material and conjugate. A substrate solution is added which results in a chromogenic reaction catalysed by the HRP conjugated to the detection antibody, giving rise to development of a blue colour. The reaction is stopped with the addition of acidic reagent and the colour is changed to yellow. The colour intensity is proportional to the concentration of ferritin in the sample and can be measured spectrophotometrically.

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 plastic sealable bag provided 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 (ng/ml) made up in a human serum matrix. The exact concentrations are provided on the vial labels. Concentrations given in the IFU are subject to change. Ready to use. Once open stable for 1 month at 2 - 8°C.
Enzyme Conjugate	1x11ml	1 vial containing HRP labelled monoclonal Anti- ferritin antibody in buffered saline. Once open, stable for 2 months at 2 - 8°C.
Wash Buffer Concentrate (40X)	1x25ml	PBS-Tween at pH 7.4. 40X concentrate. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x11ml	TMB and hydrogen peroxide reagent. Ready to use. Once open, stable for 2 months at 2 - 8°C.
Stop Solution	1x6ml	Diluted sulphuric acid solution (1M) Ready to use. Once open, stable for 2 months at 2 -8°C.

IFU, resealable bag, plate covers.

Materials required but not provided:

Distilled water, micropipettes, incubator, 96-well plate reader and 96-well plate washer, absorbent paper.

Sample Collection:

Collect serum samples by separation from red blood cells after standard venepuncture technique. Store samples at 15 - 25°C for up to 24 hours, for 7 days at 2 - 8°C and 1 month at -20°C, under which conditions ferritin will be stable with a recovery within 90-110%. Avoid more than one freeze-thaw. Some sample collection tubes may contain differing substances which could affect the test result. If samples contain precipitate centrifuge before use. Do not use heat-inactivated samples. Do not use samples and controls stabilised with azide. Avoid grossly haemolytic, lipemic or turbid samples.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored unopened 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 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 expiry if less.

Precautions:

The ELISA assay is time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure and do not modify the steps. Reliability of results cannot be guaranteed if there are deviations from the instructions.
The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.
The calibrators contain human serum based components. They have been tested and found to be non-reactive to HBsAg, HIV and HCV antibodies and syphilis. The assay contains materials of animal origin including BSA which have been sourced from countries where BSE has not been reported. However, it is recommended that all the reagents be handled as if potentially infectious and care to be taken in their use and disposal.
Wear laboratory protective equipment including gloves and safety glasses whilst handling reagents, controls and samples. Wash hands thoroughly after each operation.
Samples and reagent additions to wells should not introduce any bubbles as these may cause erroneous results.
Use new pipette tips for each sample and reagent addition to avoid cross contamination.
Do not use the kit beyond the expiry date.
Do not interchange components from other kits.

Procedure:

Reagent preparation:

Ensure the samples, calibrators, and controls are at room temperature (15 - 25°C) before beginning the assay. Mix all reagents gently before use. Prepare wash solution concentrate by adding the contents of the bottle to 975 ml distilled water or dilute a portion by 1/40. Stable for 2 months at room temperature. Do not use Substrate if it looks blue. Do not use reagents that are contaminated.

STEP 1

Preparation: Remove the number of wells required and assign each well for the calibrators, controls and samples.

STEP 2

Addition of Samples: Add 25 µl of Calibrators, controls and samples to each well.

STEP 3

Addition of Enzyme Conjugate: Add 100 µl of Enzyme Conjugate to each well. Tap the side of the plate gently to ensure that the added components are well mixed.

STEP 4

Incubation: Cover the plate with a plate cover and incubate for 60 minutes at 37°C.

STEP 5

Washing: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted wash solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. Use of an automated microplate strip washer is recommended. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 6

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

STEP 7

Incubation: Cover the plate with a plate cover and incubate for 20 minutes at room temperature (15 - 25°C). Ensure that this incubation is done in the dark.

STEP 8

Stopping the Reaction: Add 50 µl of the Stop solution into each well Tap the side of the plate gently till the solution changes to completely from blue to yellow.

STEP 9

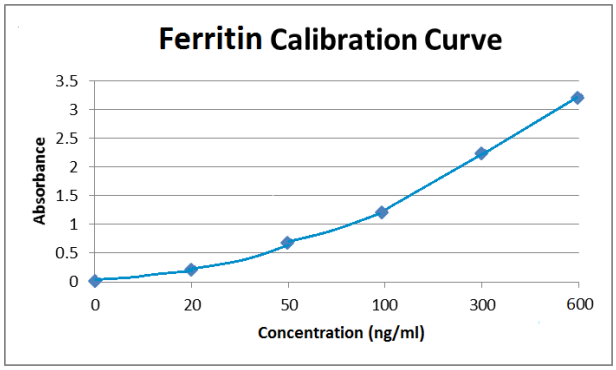
Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader within 30 minutes of adding the Stop Solution. Note down the absorbances.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance on the y axis and concentration in ng/ml on the 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 Only:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	0.015	0.0 ng/ml
CAL B	0.188	20 ng/ml
CAL C	0.635	50 ng/ml
CAL D	1.178	100 ng/ml
CAL E	2.210	300 ng/ml
CAL F	3.279	600 ng/ml



AMS UK Ltd, 42 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL. Tel: +44(0)28 25656268
www.amslabs.co.uk info@amslabs.co.uk

V3: rev Oct 2020

Limitations:
The washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances**.**
Kit failure may result from using kits beyond the expiry date, poor washing procedures, contaminated reagents, improper operation of equipment, sample collection issues or timing errors.
This kit is intended only for testing of serum samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled blood.
Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop human anti-mouse antibodies (HAMA). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
The result from this test should not be used as the sole criteria for the diagnosis of iron status or treatment, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

Performance:

Interference
The assay is unaffected by bilirubin < 1112 µmol/l (< 65 mg/dl), haemoglobin < 0.31 mmol/l (< 0.5 g/dl), lipemia (Intralipid < 1900 mg/dl) and biotin (< 205 nmol/l (< 50 ng/ml) based on Recovery within ± 10 % of initial value. No interference was observed from rheumatoid factors up to a concentration of 2500 IU/ml
Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
There is no prozone effect at ferritin concentrations up to 50000 ng/ml.
In vitro tests were performed on 19 commonly used pharmaceuticals. No interference with the assay was found.
At therapeutic concentrations Fe^{2+} and Fe^{3+} ions do not interfere in the Ferritin ELISA.
In rare cases there may be interference from exceedingly high titres of antibodies to streptavidin and ruthenium which can be minimised by appropriate test design.

Measuring range
0.5 - 1000 ng/ml (defined by the lower detection limit and the maximum of the master curve). Values outside this range should be reported as less than or greater than.

Limit of Detection
0.1 ng/ml
The detection limit represents the lowest analyte level that can be distinguished from zero.

Expected values
Men: 27 - 430 ng/ml (adult ≤ 60yr)
Women: 15 - 168 ng/ml (adult ≤ 60yr)

Precision
Precision was determined using reagents, pooled human sera, and controls testing 2 times daily for 20 days (n = 40). The following results were obtained:

ID	Intra Assay Precision		
	Mean (ng/ml)	SD	%CV
Sample 1	2.85	0.243	8.53
Sample 2	64.71	3.436	5.31
Sample 3	292.55	13.925	4.76

Method comparison
A comparison of the ferritin assay (y) with another commercially available ferritin ELISA (x) using clinical samples (n = 156) gave the following correlation:
y = 1.073 x + 0.011, r = 0.975
The sample concentrations were approximately between 0 - 964 ng/ml.

Analytical specificity
Human liver ferritin 98% recovery.
Human spleen ferritin 90% recovery.
Human heart ferritin 1% recovery.

References

1. Wick M, Pinggera W and Lehmann P. In Iron Metabolism: Diagnosis of anaemias (2nd ed). Springer-Verlag, 1995 ISBN 3-211-82525-8.
2. Arosio P et al. Heterogeneity of ferritin II: Immunological aspects. In: Albertini A et al (eds). Ferritins and isoferritins as biochemical markers. Elsevier, Amsterdam 1984:33-47.
3. Morikawa K et al. A role for ferritin in haematopoiesis and the immune system. Leuk-Lymphoma, 1995: 18:429-433.
4. Cook JD et al. Iron deficiency: the global perspective. Ad Exo Ned Biol, 1994; 356:219-228.
5. Jacobs A et al. Functional aspects of isoferritins. In: Albertini A et al (eds). Ferritins and isoferritins as biochemical markers. Elsevier, Amsterdam 1984:113-127.

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

CE

AMS UK Ltd, 42 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL. Tel: +44(0)28 25656268
www.amslabs.co.uk info@amslabs.co.uk

V3: rev Oct 2020