BIOCLONE

ELEGANCE
FERRITIN ELISA KIT

REF 40 470096

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WARRANTY

The manufacturer makes no express warranty other than the diagnostic kit will measure the designated analyte when used in accordance with the manufacturer's printed instructions. The use of the diagnostic kit for any other purpose is outside the intended use of this product and is done at the user's own risk.
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INTENDED USE

The *ELEGANCE* Ferritin ELISA has been designed for the quantitative *in vitro* diagnostic measurement of human Ferritin in serum or plasma.

PRINCIPLES OF THE *ELEGANCE* ELISA

The *ELEGANCE* ELISA is an enzyme-linked immunoassay. The sample antigen is "sandwiched" between the antibody bound to the microwell and the biotinylated antibody reagent. The microwells are washed to remove any unbound material. Streptavidin-peroxidase (Amplification Reagent) is added and binds to the biotinylated antibody at many sites. After washing, the substrate solution reacts with any bound peroxidase to produce colour in direct proportion with the amount of sample antigen, which can be calculated from the calibrator curve.

***ELEGANCE* REAGENTS PROVIDED, STABILITY AND STORAGE**

Kit size - 96 tests. The kit and all its components, unopened or opened, should be stored at 2-8°C until the listed expiry dates.

Ferritin:
Coated Microwells
96 wells REF # FEA96
Frame containing microwells coated with anti-Ferritin antibody. Ready to use.

Ferritin: Antibody Reagent
1 vial REF # FEB96
10 mL biotinylated anti-Ferritin antibody in a buffered solution containing bovine serum albumin, non-immune animal sera and a blue dye. Contains sodium azide, 0.2% w/v and thiomersal, 0.01% w/v. Ready to use.

Ferritin:
Amplification Reagent
1 vial REF # FEP96
10 mL streptavidin-peroxidase (streptavidin from *S. avidinii*) in a buffered solution containing bovine serum albumin and a violet dye. Contains Bronidox L, 0.2% v/v and thiomersal, 0.02% w/v. Ready to use.

Wash Concentrate
1 vial REF # EWC96
50 mL of a 15 x concentrated wash solution. Contains thiomersal, 0.09% w/v. To be diluted before use.

Substrate Buffer
1 vial REF # ESB20
20 mL urea peroxide in a citrate-phosphate buffer. Contains thiomersal, 0.01% w/v.

Substrate Tablets
1 vial REF # EST4
4 x 4 mg tablets of ortho-phenylenediamine (OPD) with inactive ingredients.

Ferritin: Calibrators
6 vials REF # EFES1-6
2.0 mL in Calibrator A and 0.5 mL in Calibrator B-F each in 5% BSA PBS buffer. Contains sodium azide, 0.1% w/v. Ready to use.

PRECAUTIONS AND WARNINGS TO USERS

Handling of specimens and kit components, their use, storage and disposal should be in accordance with any local or national laboratory safety procedures or regulations.

Specimens and Calibrators

The source material of the calibrators has been tested by an approved accredited method for the presence of hepatitis B surface antigen, antibody to hepatitis C and antibody to HIV - 1/2 (AIDS) and has been found to be non-reactive for all. However, it is recommended that all samples be handled as if capable of transmitting infectious disease.

Preservatives

The kit contains sodium azide, thiomersal and Bronidox L as preservatives. As reagents contain potentially toxic preservatives care should be taken in handling to avoid ingestion or skin contact. Sodium azide may react with lead and copper plumbing to form potentially explosive azides.

Substrate

Avoid any skin contact.

SPECIMEN COLLECTION AND HANDLING

No special patient preparation is required. Specimens can be either serum or plasma collected in a manner appropriate for laboratory testing. Serum is preferred, however the anticoagulant heparin can be employed without sacrificing accuracy. Avoid grossly haemolytic, lipaemic and turbid specimens. Specimens can be stored at 2-8°C for up to 48 hours. Specimens held for longer should be stored at or below -20°C. Specimens should not be frozen and thawed repeatedly. Thawed specimens should be checked for flocculent matter and mixed by inversion just prior to testing.
Turbid specimens or specimens containing particulate matter should be centrifuged prior to use.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

* Distilled or deionised water
* 1M H_2SO_4
* Precision pipettes
* Repeating pipette
* 1L measuring cylinder
* Absorbent tissue (lint-free)
* Timer
* Vortex mixer
* Microtitre plate shaker
* Microtitre plate washer
* Microplate reader system.

PROCEDURAL NOTES

Bring all reagents and specimens to room temperature (20-25°C) and mix by gentle inversion prior to use. Duplicates are recommended. Contamination of reagents will lead to poor performance. A calibrator curve should be run with each assay. Specimens suspected of having concentrations above the top calibrator should be diluted in zero calibrator before assay. All assay steps should be performed without interruption, but if the wells cannot be filled with Amplification Reagent or substrate solution immediately after washing, then the microwells may be left upside down on absorbent lint-free tissue for a maximum of 15 minutes.
Reagents are matched in each kit and therefore reagents from different lot numbers should not be mixed.
The photometer and all pipettes used should be calibrated appropriately before use.

Washing

The efficiency of the wash step is vital for good precision. Mircowells are washed using an automatic plate washer. Avoid overflows from one well to another.

Quality Control

Control specimens should be run in every assay to ensure correct procedure. Control values should lie within laboratory ranges before assay is approved.

ASSAY PROCEDURE

Preparation of Reagents

Wash Solution

Dilute the wash concentrate 1 in 15 with deionised water. The wash solution can be stored at room temperature (20-25°C) for 12 weeks.

Substrate Solution

It is recommended that this reagent be made up just prior to use. Place correct number of OPD tablets into the required amount of Substrate Buffer. Add 1 tablet per 5 mL. After tablets have completely dissolved (1-2 minutes) and no bubbles remain, replace stopper on

bottle and mix by inversion. Avoid strong light. The substrate solution must be used within 30 minutes of preparation.

Calibrators

Mix the vials by gentle inversion. Exact concentrations determined lot-to-lot are stated on a separate label inside the kit. The calibrators can be stored at 2-8°C.

Protocol

1. Assemble the microwells in the frame according to the number of tests required. Bag and return unused wells to 2-8°C.

2. Pipette 25 µL of sample (calibrator, control, specimen) in duplicate into the appropriate wells. Time taken to dispense the samples should not exceed 20 minutes.

3. Pipette 100 µL of Ferritin Antibody Reagent (blue) into all wells.

4. Cover microwells with lid and incubate for 60 minutes on a plate shaker at room temperature (20-25°C).

5. After incubation, wash the microwells. Aspirate the liquid and rinse each well 4 times with 250 µL wash solution. After the final wash, invert the microwells and tap firmly on absorbent tissue to remove any remaining wash solution. Ensure that no air bubbles remain in the wells.

6. Pipette 100 µL of Ferritin Amplification Reagent (violet) into all wells.

7. Cover microwells with lid and incubate for 10 minutes on a plate shaker at room temperature (20-25°C).

8. After incubation, repeat wash step.

9. Pipette 100 µL of prepared substrate solution into all wells. Timing of the incubation step is measured from the addition of substrate solution to the first well.

10. Cover microwells with lid and incubate for 5 minutes stationary at room temperature (20-25°C).

11. Pipette 50 µL of 1M H_2SO_4 into all wells in the same timed sequence as for substrate solution addition.

12. Carry out an end-point reading at 490 nm and process data as described in the microplate reader user's manual. This reading step should be carried out within 30 minutes of stopping the reaction.

CALCULATION OF RESULTS

Calculation of results can be carried out manually if there is no automatic data reduction. Determine the OD for each well. Plot the calibrator curve using log-log graph paper with concentration of calibrators on the x-axis and OD on the y-axis. The curve may be drawn point-to-point or a curve-fitting routine, such as spline interpolation, may be used. Interpolate the sample values from OD measured from this calibrator curve. Record the value for each sample in µg/L Ferritin.

The range of the *ELEGANCE* Ferritin ELISA is from 0 to approx. 1000 µg/L, but the maximum concentration that can be reported is limited by the linear performance characteristics of the photometer used.

If the OD value of the highest calibrator is above the range of the photometer, then this calibrator must be omitted from the plot of the calibrator curve.

MODEL CALCULATIONS

Endpoint Data

ID	Ave OD	Ferritin (µg/L)
0	0.043	
5	0.072	
20	0.148	
100	0.696	
300	1.723	
1000	2.951	
Sample 1	1.453	228.5
Sample 2	0.915	136.0
Sample 3	0.410	61.0

CALIBRATION

The calibrators supplied in this kit are calibrated and labelled in µg/L, referenced to the WHO 1st IS 80/602.

LIMITATIONS

Serum specimens showing gross haemolysis, gross lipaemia, or turbidity may give false results.

Specimens from patients who have high circulating anti-mouse antibodies as a result of mouse monoclonal antibody therapy may give falsely elevated or depressed levels. These specimens should not be assayed using this kit.

EXPECTED VALUES

It is recommended that each laboratory establish its own reference range based on a representative sample population. The following reference range was obtained by assaying serum samples from healthy individuals and is given as a guide only:

		n	Mean (µg/L)	Ferritin Range (µg/L)
Female	< 45 years	89	35.8	4.4 - 135.9
	≥ 45 years	59	57.8	13.2 - 165.7
Male		182	87.6	8.6 - 313.9

PERFORMANCE CHARACTERISTICS

Intra-assay Precision

Sample	n	Mean ± 2SD (µg/L)	%CV
1	16	71.2 ± 5.4	3.9
2	16	146.6 ± 14.5	5.0
3	16	303.3 ± 23.6	3.9

Inter-assay Precision

Sample	n *	Mean ± 2SD (µg/L)	%CV
1	21	64.3 ± 10.1	7.9
2	21	142.8 ± 19.7	6.9
3	21	332.0 ± 35.5	5.4

* duplicate

Specificity

The antibodies in the *ELEGANCE* FERRITIN ELISA were tested for their specificity and were found to be 100% cross reactive for both liver and spleen Ferritin.

Accuracy

Recovery was calculated by assaying before and after addition of exogenous analyte.

Sample	Ferritin (µg/L) Observed	Ferritin (µg/L) Expected	% Recovery
1	50.3	47.9	105.0
2	99.8	97.7	102.1
3	184.4	186.6	98.8
4	262.2	284.6	92.1

Dilution

A sample was diluted in zero calibrator, assayed and recovery calculated.

Sample	Ferritin (µg/L) Observed	Ferritin (µg/L) Expected	% Recovery
Neat	176.6		
1/2	93.7	88.3	106.1
1/4	45.8	44.2	103.6
1/8	22.2	22.1	100.0
1/16	11.0	11.0	100.0

High-dose Hook Effect

Due to the high-dose hook effect characteristic of the assay, samples greater than 50,000 µg/L may yield aberrant results, less than that of the kit's highest calibrator. Those samples should be diluted with the zero calibrator and reassayed.

Sensitivity

The sensitivity of the assay is typically less than 2 µg/L.

The sensitivity is defined as the concentration of analyte which corresponds to the dose response variable (OD) that is two standard deviations from the mean dose response variable of 16 replicate determinations of the zero calibrator run in three different assays.

Interference

No interference with analyte recovery was observed for concentrations of haemoglobin up to 250 mg/dL, bilirubin up to 10 mg/dL and triglycerides up to 970 mg/dL.

ORDERING INFORMATION

The *ELEGANCE* FERRITIN ELISA is manufactured by:

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