KRISHGEN PUDGALA
PARTNER FOR PRECISION DIAGNOSTICS

Ferritin GENLISA® ELISA

REF : KBD1025

Ver 1.5

IVD

Enzyme Immunoassay for the Quantitative Determination of Ferritin in serum and plasma

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

REF **KBD1025** **96 tests**

Krishgen Pudgala LLP Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400018. India.
Tel: +91-22-49198700 | email: sales@krishgenpudgala.com

Introduction:

Ferritin is major iron storage protein in the human body with a molecular weight of 450 kilo Daltons (kDa). Ferritin is found in various tissues including liver, spleen, brain and bone marrow and its molecule composed of 24 protein subunits which constitute a central hollow sphere filled by at least 4000 soluble iron atoms. The iron which is bound to Ferritin, constitutes 1% of total plasma iron and comprises most important and highly specific storage form of iron in cells. The bound iron is quickly released from the Ferritin once iron deficiency status occurred. Ferritin level is high at birth but gradually reduced during the first few months and remains low in childhood. In puberty, Ferritin level raised steadily and in adults, men have higher levels than women. Serum Ferritin is a good indicator of iron storage and its level remains constant during the biorhythm in contrast to alternating iron values. Thus serum Ferritin level is a good reflection of actual iron storage of the body and a reliable indicator for iron deficiency as well as iron overload status. Ferritin level determination is an important parameter for the diagnosis and therapy control of the iron deficiency and it is also recommended for risk groups like blood donors, pregnant women, hemodialysis patients and infants. In hemochromatosis, anemia of chronic disease, hemolytic anemia like thalassemia, liver disease, malignancies and inflammation, high levels of serum Ferritin have been described.

Intended Use:

The Ferritin GENLISA® ELISA is intended for the quantitative and semi-quantitative detection of antibodies to double-stranded DNA (Ferritin) in serum and plasma samples.

Principle:

The Ferritin GENLISA® ELISA Quantitative test kit is based on a solid phase enzyme-linked immune sorbent assay. The Microtiter wells are pre-coated with specific Ferritin antibodies. Standards, Samples and Controls are pipetted into microwells and Ferritin present in sample binds to the antibody coated on the wells. Enzyme Conjugate antibody is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of Ferritin present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Ferritin Antibody Microtiter Coated Plate (96 wells) - 1 no
2. Ferritin Standard 0 - 2ml
3. Ferritin Standard (10, 25, 100, 200, 400 and 800 ng/ml) – 1ml/vial
4. Low Control Serum – 1 ml
5. High Control Serum – 1 ml
6. Assay Buffer – 12 ml
7. Enzyme Conjugate – 12 ml
8. (20X) Wash Buffer – 50 ml
9. TMB Substrate - 12 ml
10. Stop Solution - 12 ml
11. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipette to measure volumes ranging from 10 ul, 100 ul to 1000 ul.
3. Deionized (DI) water.
4. Distilled Water.
5. Graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.
4. Do not freeze.
5. Protect from light and moisture.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.

2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, re-centrifuge.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 50 ml of Wash Buffer (20X) to 950 ml of DI water. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **50 ul Standard, Control Serum and specimen** in appropriate wells.
3. Add **100 ul Assay Buffer** into the wells and shake gently for 15 seconds.
4. Seal the plate and Incubate at room temperature for 30 minutes.
5. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
6. Add **100 ul** of **Enzyme Conjugate** to each well.
7. Seal the plate and Incubate at room temperature for 30 minutes.
8. Repeat step as No. 5.
9. Add **100 ul** of **TMB Substrate** into each well.
10. Incubate at room temperature for **15 minutes** in dark.
11. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 30 minutes of stopping the reaction.

Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Using semi-log graph paper or computer programs, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit straight line through the standard points. To determine the unknown concentrations, find the unknowns mean absorbance value on the y-axis and draw a horizontal line to the standard curve.

At the point of intersection, draw a vertical line to the x-axis and read the Carbamazepine concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software; 4-PL or cubic spline or polynomial regression (2nd order) may be preferred.

Expected Control Values:

High Control Serum: 260 – 530 ng/ml
Low Control Serum: 9.5 – 17.5 ng/ml

Expected Values

It is important for each laboratory to establish the normal range limits. The following reference range from healthy persons should be considered as a guideline only:

	Reference interval (ng/ml)
Adult Male	20-300
Pre-menopause females	10-100
Post-menopause female	20-200
Infants	150-500

Performance Characteristics:

1. Minimum Detection Limit
The minimum detectable concentration of Ferritin by this assay is estimated to be 1 ng/ml.

2. Test Precision
Intra, as well as Inter-assay precision, carried out by 3 different sera, and the results are shown in tables 1 and 2:

Intra-assay:

No.	No. of tests	Mean (ng/ml)	SD (ng/ml)	CV%
1	24	16.5	0.60	3.6
2	24	365	11.7	3.2
3	24	585	32.0	5.4

Inter-assay:

No.	No. of tests	Mean (ng/ml)	SD (ng/ml)	CV%
1	10	20.3	1.20	5.9
2	10	372	18.7	5.0
3	10	566	52.6	9.3

*Each test was run in duplicate.

Recovery
The certain amounts of Ferritin were added into 4 different sera with known concentrations of Ferritin and then their recovery were determined. The results shown below:

No.	Serum Ferritin level (ng/ml)	Ferritin added (ng/ml)	Exp. (ng/ml)	Obs. (ng/ml)	Rec. (%)
1	12	10	11	10.5	95
1	12	100	56	54	96
1	12	500	256	250	97
2	44	10	27	29	107
2	44	100	72	74	103
2	44	500	272	280	103
3	152	10	81	78	96
3	152	100	126	130	103
3	152	500	326	321	98
4	416	10	213	270	103
4	416	100	258	301	98
4	416	500	458	469	102

Exp: Expected, Obs: Observed,
Rec.: Recovery

Linearity
To verify test linearity, three different serum samples with known Ferritin concentration were diluted sequentially by Sample Diluent. Then the sera were tested by the ELISA. The results of serum recovery were determined considering dilution factor.

		Recovery (%)			
No	**Ferritin (ng/ml) undiluted specimen**	**1:2**	**1:4**	**1:8**	**1:16**
1	550	106	98	101	91
2	370	112	101	96	83
3	200	109	105	95	97
4	94	98	101	103	90

Hook Effect
To rule out possible hook effect occurrence, the Ferritin Assay was done on serums with high concentration of Ferritin (up to 250 ug/ml) and no “Hook effect” was seen.

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material

- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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Krishgen Pudgala LLP, 2026.

THANK YOU FOR USING KRISHGEN PRODUCT!

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EC REP

Unit No.1/2, Om Sainath Commercial Complex,
Off Mankoli-Anjur Phata Road. Village Dapode, Bhiwandi 421302.
KinesisDx, Lyoner Strasse 14, Frankfurt, Germany

Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **50 ul Standard, Control Serum and specimen** in appropriate wells.
3	Add **100 ul Assay Buffer** into the wells and shake gently for 15 seconds.
4	Seal the plate and Incubate at room temperature (22-28ºC) for 30 minutes.
5	Aspirate and wash plate 5 times with (1X) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
6	Add **100 ul** of **Enzyme Conjugate** to each well.
7	Seal the plate and Incubate at room temperature (22-28ºC) for 30 minutes.
8	Repeat step as No. 5.
9	Add **100 ul of TMB Substrate** into each well.
10	Incubate at room temperature for 15 minutes in dark.
11	Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction.

SYMBOLS KEY

MTP	Microtiter Plate (96wells)
STD	Standard
Low CNTRL SERUM	Low Control Serum
HIGH CNTRL SERUM	High Control Serum
ASY BUF	Assay Buffer
ENZY CONJ	Enzyme Conjugate
20X WASH BUF	(20X) Wash Buffer
1X ASY BUF	(1X) Assay Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature