# Human Ferritin GENLISA™ ELISA

REF: KBD1025

Ver 1.1

**IVD**

Enzyme Immunoassay for Quantitative Determination of Ferritin From Human Serum

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

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**REF KBD1025** **Σ 96 tests**

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**Introduction:**

One of the most prevalent disorders of man is the dietary deficiency of iron and the resulting anemia. Therefore, the assays of iron, total iron binding capacity, and other assessments of iron compounds in the body are clinically significant. Iron-storage compounds in the body include hemoglobin, hemosiderin, myoglobulin, and the cytochromes. In most tissues, ferritin is a major iron-storage protein.

The measurement of ferritin in serum is useful in determining changes in body iron storage, and is non-invasive with relatively little patient discomfort. Serum ferritin levels can be measured routinely and are particularly useful in the early detection of iron-deficiency anemia in apparently healthy people. Serum ferritin measurements are also clinically significant in the monitoring of the iron status of pregnant women, blood donors, and renal dialysis patients. High ferritin levels may indicate iron overload without apparent liver damage, as may be noted in the 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.

**Intended Use:**

Human Ferritin GENLISA™ ELISA is specifically designed for the Quantitative determination of Ferritin from Human Serum.

**Principle:**

Human Ferritin GENLISA™ ELISA is a sandwich enzyme linked immnunosorbent assay which is designed to quantitatively detect ferritin antigen present in human serum. Anti-ferritin antibody is pre-coated onto microwells. Standards and Samples are pipetted into microwells and sample present binds to the antibody coated on the wells. And then enzyme labeled Anti-ferritin HRP conjugate is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the TMB Substrate Solution is added to microwells and color develops proportionally to the amount of Human Ferritin present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

**Materials Provided:**

1. Anti- Ferritin antibody Coated Microtiter Plate (96 wells) - 1 no.
2. Human Ferritin Standard (0.5 ml/vial) - 0, 15, 80, 250, 500, 1000 ng/ml
3. Anti-Ferritin:HRP Conjugate - 13 ml
4. (20X) Wash Buffer – 25 ml
5. TMB Substrate – 12 ml
6. Stop Solution – 12 ml
7. Instruction Manual

**Materials to be provided by the End-User:**

1. Microplate Reader able to measure absorbance at 450nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

**Storage Information:**

1. Store the unopened kit at 2-8°C and when it is not in use, until the expiration shown on the kit label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

**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:**

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantification.

*Serum:* Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at temperature < -20°C. Avoid repeated freeze/thaw cycles.

**Reagent Preparation:**

1. Bring all reagents to Room Temperature before use.
2. To make **Wash Buffer (1X)**, dilute **25ml** of **Wash Buffer (20X)** in **475 ml** of **DI water.**

**Assay Procedure:**

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicates. A standard curve is required for each assay.

2. Add **20 ul** of **Standards** and **Samples** to the respective wells.

3. Add **100 ul** of **Anti-Ferritin:HRP Conjugate** to all wells. Gently mix for 30 seconds.

4. Incubate for 45 minutes at Room Temperature (18-25°C).

5. Aspirate and wash plate 5 times with **Wash Buffer (1X)** 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 **TMB Substrate** solution and incubate in the dark for 20 minutes at Room Temperature. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

7. Stop reaction by adding **100 ul** of **Stop Solution** to each well. Positive wells should turn from blue to yellow.

8. Read absorbance at 450 nm within 15 minutes of stopping reaction.

**Calculation of Results:**

Determine the mean absorbance for each set of duplicate standards and samples. Plot the standard curve on standard graph paper, with Ferritin concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown Ferritin 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 Ferritin concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software may be preferred. Software which is able to generate a cubic spline curve-fit or a polynomial regression to the 2nd order is best recommended for automated results.

Note:

It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the sample reads more than 800ng/ml, then dilute with Standard 0 at a dilution of not more than 1:8. Theobtained result should be multiplied by the dilution factor

If the determination value is higher or lower than normal range, it means there is an abnormal result. The final result should be diagnosed in correlation with the clinical symptoms and other diagnostic methods.

**Reference Value:**

| | |
|---|---|
| Male | 20-250 ng/ml |
| Female | 10-120 ng/ml |
| Children 6 mon. to 15 yr. | 7-140 ng/ml |

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

**Limitations of Method:**

Any clinical diagnosis should not be based on the results of in-vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

**Performance Characteristics:**

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

**Sensitivity:**
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to **5 ng/ml.**

**Accuracy:**
A statistical study using 98 healthy patient samples, ranging in ferritin concentration from 1 ng/mL to 831 ng/mL, demonstrated good correlation with a commercially available kit as shown below.

Comparison between the Human Ferritin GENLISA™ ELISA Test Kits and the Abbott AxSYMÒ Ferritin MEIA kit provided the following data:
N = 98
Correlation coefficient = 0.999 Slope = 0.993
Intercept = 1.013
Krishgen Mean = 165.5 ng/mL Abbott Mean = 165.4 ng/mL

**Specificity:**
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for human ferritin. The following hormones were tested for cross-reactivity:

| Hormone Tested | Concentration | Total Ferritin (Liver) in Serum (ng/ml) |
|---|---|---|
| Human Serum Albumin | 2.50 gm/dl | 0 |
| | 5.00 gm/dl | 0 |
| | 10.00 gm/dl | 0 |
| Alpha-Fetoprotein | 1,000 ng/ml | 0 |
| | 4,000 ng/ml | 0 |
| | 8,000 ng/ml | 0 |
| Human Hemoglobin | 125 mg/dl | 0 |
| | 250 mg/dl | 0 |
| | 500 mg/dl | 0 |
| Human Transferrin | 1.0 mg/dl | 0 |
| | 10 mg/dl | 0 |
| | 100 mg/dl | 0 |
| Ferric Chloride | 1.0 mg/dl | 0 |
| | 10 mg/dl | 0 |
| | 100 mg/dl | 0 |

**Assay Range:**
0 ng/ml to 1000 ng/ml.

**Precision:**
Intra-Assay Precision
Within-run precision was determined by replicate determinations of three different serum samples in one assay. Within-assay variability is shown below:

| Serum Sample | 1 | 2 | 3 |
|---|---|---|---|
| Number of Replicates | 24 | 24 | 24 |
| Mean Ferritin (ng/mL) | 341.6 | 231.3 | 40 |
| Standard Deviation | 12.2 | 12.1 | 1.4 |
| Coefficient of Variation (%) | 3.60% | 5.70% | 3.50% |

Inter-Assay Precision
Between-run precision was determined by replicate measurements of three different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

| Serum Sample | 1 | 2 | 3 |
|---|---|---|---|
| Number of Replicates | 24 | 24 | 24 |
| Mean Ferritin (ng/mL) | 340.1 | 220.6 | 37.3 |
| Standard Deviation | 14.3 | 11.3 | 2.5 |
| Coefficient of Variation (%) | 4.20% | 5.10% | 6.60% |

**Recovery and Linearity:**
Recovery
Various patient serum samples of known ferritin levels were combined and assayed in duplicate. The mean recovery was 98.0%

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Human Ferritin and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

| Expected Concentration (ng/mL) | Observed Concentration (ng/mL) | % Recovery |
|---|---|---|
| 744.4 | 745.1 | 100.10% |
| 350.1 | 341.6 | 97.60% |
| 165.8 | 156.0 | 94.10% |
| 84.9 | 82.5 | 97.20% |
| 39.3 | 38.9 | 99.10% |
| 20.4 | 19.4 | 95.20% |
| 10.6 | 10.2 | 96.10% |
| | | **Mean: 97.1%** |
| 753.7 | 765.6 | 101.60% |
| 374.3 | 371.1 | 99.10% |
| 182.9 | 177.3 | 96.90% |
| 91.0 | 94.5 | 103.90% |
| 46.7 | 46.5 | 99.40% |
| 24.1 | 23.3 | 96.30% |
| 12.3 | 11.7 | 95.20% |
| | | **Mean: 98.9%** |

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Human Ferritin and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

| #1. | | | |
|---|---|---|---|
| **Dilution** | **Expected Conc. (ng/ml)** | **Observed Conc. (ng/ml)** | **% Expected** |
| Undiluted | --- | 672.9 | --- |
| 1:2 | 336.5 | 310.1 | 92.20% |
| 1:4 | 168.2 | 159.3 | 94.70% |
| 1:8 | 84.1 | 84.6 | 100.50% |
| 1:16 | 42.1 | 43.0 | 102.30% |
| 1:32 | 21.0 | 22.1 | 105.10% |
| 1:64 | 10.5 | 11.6 | 110.30% |
| | | | Mean = 100.9% |

| #2. | | | |
|---|---|---|---|
| **Dilution** | **Expected Conc. (ng/ml)** | **Observed Conc. (ng/ml)** | **% Expected** |
| Undiluted | --- | 828.1 | --- |
| 1:2 | 414.1 | 450.2 | 108.70% |
| 1:4 | 207.0 | 223.5 | 108.00% |
| 1:8 | 103.5 | 115.2 | 111.30% |
| 1:16 | 51.8 | 53.9 | 104.20% |
| 1:32 | 25.9 | 27.7 | 107.10% |
| 1:64 | 12.9 | 15.0 | 116.40% |
| | | | Mean = 109.2% |

| #3. | | | |
|---|---|---|---|
| **Dilution** | **Expected Conc. (ng/ml)** | **Observed Conc. (ng/ml)** | **% Expected** |
| Undiluted | --- | 423.8 | --- |
| 1:2 | 211.9 | 209.3 | 98.80% |
| 1:4 | 106.0 | 102.5 | 96.70% |
| 1:8 | 53.0 | 52.2 | 98.40% |
| 1:16 | 26.5 | 26.8 | 101.00% |
| 1:32 | 13.2 | 14.3 | 100.60% |
| | | | Mean = 100.6% |

**Hook Effect:**
No high dose hook effect is observed in this assay at ferritin levels up to 12,000 ng/ml.

**Standardization:**
The Reference Standards are calibrated against the International Committee for Standardization in Hematology ICSH) Expert Panel on Iron, human liver standard (NIBSC-WHO 80/602).

**Quality Control:**
Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges.

**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 human 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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**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 **20 ul of Standards** and **Sample** into appropriate wells. |
| 3 | Add **100 ul** of **Anti-Ferritin:HRP Conjugate** into each well. Mix well. Incubate at room temperature for **45 minutes** |
| 4 | 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. |
| 5 | Add **100 ul** of **TMB Substrate** to all wells. |
| 6 | Incubate on shaker at **room temperature** for **20 minutes.** |
| 7 | Add **100 ul** of **Stop Solution**. **Read result with an ELISA reader at 450 nm** within 15 minutes of stopping the reaction. |

## SYMBOLS KEY

| | |
|---|---|
| MTP | **Coated Microtiter Plate (96 wells)** |
| STD | **Standard** |
| HRP CONJ | **HRP Conjugate** |
| SUB TMB | **TMB Substrate** |
| SOLN STOP | **Stop Solution** |
| 20X WASH BUF | **(20X) Wash Buffer** |
|  | **Consult Instructions for Use** |
| REF | **Catalog Number** |
|  | **Expiration Date** |
|  | **Storage Temperature** |