TRUSTwell®

Ferritin ELISA Kit

REF

AE5010

IVD

- 96-well ELISA kit for the quantitative detection of Ferritin concentration in human serum or Plasma
- Store at 2-8°C upon receipt

INTENDED USE

The TRUSTwell Ferritin ELISA is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of ferritin (FTN) concentration in human serum. It is intended to be used by professionals only.

INTRODUCTION

Iron is an essential element that is present in all cells in the human body. It is an important component of hemoglobin and is required for oxygen transport and electron transfer reactions1. Most of the iron in the body is found in red blood cells, and to a lesser extent in muscle and cellular enzymes2. The leftover iron is stored until it is needed. Ferritin is a 24-subunit, intracellular protein core that functions to store and release iron. Under normal circumstances, serum ferritin concentration is positively correlated with the total amount iron stores in the body1-3. Normal serum ferritin concentrations vary with age and sex. If serum ferritin concentration is low, however, it usually indicates iron deficiency2-4. Iron deficiency is one of the major causes of anemia, which is characterized by low levels of hemoglobin and functioning red blood cells. Anemia is most prevalent in highly susceptible populations, which include pregnant women and children3. If serum ferritin concentration is high, it may indicate iron overload. Alternatively, elevated ferritin may be the result of a variety of other factors, including high body mass index (BMI), aging (post-menopausal women), or the presence of inflammation, infection, certain malignancies, or liver or kidney disease2-4. Thus, high serum ferritin concentrations usually require further investigation1,3. The TRUSTwell Ferritin ELISA kit is intended to be used for the quantitative measurement of ferritin in human serum. Measurement of iron status by serum ferritin is important for accurate classification of iron status in individuals, in order to provide treatment for iron deficiency or to initiate further testing and a plan of care for elevated serum ferritin concentrations2-4. Determination of iron status in populations is also important to determine geographical prevalence and develop public health intervention, if necessary.

TEST PRINCIPLE

The TRUSTwell Ferritin ELISA kit is a solid-phase enzyme-linked immunosorbent assay based on the principle of the double antibody sandwich technique for the determination of FTN concentration in human serum.

The TRUSTwell Ferritin ELISA kit is comprised of five key components:

1) Solid microwells pre-coated with streptavidin
2) FTN Calibrators
3) FTN Assay Control
4) FTN Biotin Reagent comprised of anti-FTN conjugated to biotin (Bio-Ab) and
5) FTN Enzyme Reagent comprised of anti-FTN conjugated to HRP enzyme (HRP-Ab)

During the assay, the test specimen and FTN Biotin Reagent are added to the microwell precoated with Streptavidin. During the first incubation, the biotin in the Bio- Ab binds to the streptavidin coated on the microwell surface, and the anti-FTN antibody binds to the FTN in the test specimen. After a wash step, FTN Enzyme Reagent is added to the microwell. During the second incubation, the anti-FTN in the HRP-Ab binds to the FTN in the patient specimen.
After another wash step to remove excess conjugate and serum protein, the TMB substrate is added to the microwell, which reacts with the HRP in the HRP-Ab during the third incubation. The presence of the conjugate is shown by the development of a blue color. The reaction is then terminated by the addition of Stop Solution into the microwell, and the absorbance is determined using a spectrophotometer at 450/610- 640 nm. The color development is directly proportional to the concentration of FTN in the calibrators, controls, and test specimens. A graph can then be plotted using calibrator FTN concentrations on the x-axis and the relative absorbance values on the y-axis. By interpolating the absorbance, the concentrations for unknown samples can be determined.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Streptavidin Coated Microwells	8 wells x 12 strips	AE5010W
2	FTN Calibrators: C1 (0 ng/mL)	0.25 mL	AE5010C1
3	C2 (10 ng/mL)	0.25 mL	AE5010C2
4	C3 (50 ng/mL)	0.25 mL	AE5010C3
5	C4 (150 ng/mL)	0.25 mL	AE5010C4
6	C5 (400 ng/mL)	0.25 mL	AE5010C5
7	C6 (800 ng/mL)	0.25 mL	AE5010C6
8	FTN Assay Control	0.25 mL	AE5010AC
9	FTN Enzyme Reagent	12 mL	AE5010H
10	FTN Biotin Reagent	12 mL	AE5010B
11	Wash Buffer Concentrate (40x)	25 mL	AWE3000
12	TMB Substrate A	6 mL	ATME2000A
13	TMB Substrate B	6 mL	ATME2000B
14	Stop Solution	7 mL	ASE1000
15	Instructions for Use	1	PI-AE5010
16	ELISA Working Sheet	2	AE0001ES
17	Microplate Sealers	3	N/A

Materials and reagents required but not provided in the kit

1. Micropipettes capable of delivering 10 µL, 50 µL, 100 µL, and 1 mL 1.5mL and 10 mL micro tube
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-2.5 or greater at 450 nm wavelength is acceptable
3. Vortex mixer or equivalent
4. Absorbent paper for blotting the microwells
5. Graph paper
6. Timer
7. Distilled or de-ionized water

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. Ensure that the reagents are brought to room temperature before testing. After removing the desired number of wells, place unused wells in the resealable plastic bag with the desiccant provided and return to 2-8°C. All reagents are stable through the expiration date printed on the label if not opened. Once opened, the kit is stable for 8 weeks at 2-8°C, or until the labeled expiration date, whichever is earlier.

SPECIMEN COLLECTION AND PREPARATION

- Serum specimens should be prepared from whole blood obtained by acceptable venipuncture technique.
- If not tested immediately, the specimens can be stored at 2-8°C for up to 7 days. The specimens should be frozen at -20°C for longer storage. Avoid multiple freeze-thaw cycles. If a specimen is to be shipped, pack in compliance with federal regulations covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation before testing.
- Do not use specimens demonstrating gross lipemia, gross hemolysis or turbidity.
- Specimens containing sodium azide may interfere with test results

PREPARATION OF THE REAGENTS

1. Bring all reagents to room temperature (20-28°C).
2. Preparation of working Wash Buffer:

If precipitants are visible, warm up the Wash Buffer (40X concentrate) at 37°C.
Dilute concentrated Wash Buffer 40-fold with water as follows:

Plate	DI water	40X wash buffer	Final volume
Full plate	195 mL	5 mL	200 mL
Half plate	97.5 mL	2.5 mL	100 mL
Quarter plate	48.75 mL	1.25 mL	50 mL

3. Mix each reagent before adding to the test wells.
4. Determine the number of strips needed and mark on the ELISA working sheet with the appropriate information. Calibrators should be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Calculate the desired number of microwells. Remove the remaining microwells and place them with desiccant into the resealable plastic bag, seal and store at 2-8°C for later use.
2. Add 25 µL of FTN calibrators, FTN Assay Control, and patient specimens into the assigned wells, respectively.
3. Add 100 µL of FTN Biotin Reagent into each well.
4. Cover the plate with a microplate sealer, and then shake gently for 10 seconds.
5. Incubate the microplate at room temperature (20-28°C) for 30 minutes.
6. Wash Step (Can be performed manually or with automated washing):**Manual washing:** Carefully remove the incubation mixture by disposing the solution into a waste container. Fill each well with 350 µL working wash buffer and shake gently for 20-30 seconds. Discard the wash solution completely. Repeat 2 more times. After completing the last wash step, tap the plate on absorbent paper to remove residual liquid. **Automated washing:** Automatic plate washer must be calibrated to ensure efficient washing. Fill each well with 350 µL diluted wash buffer. Aspirate all wells completely. Repeat 2 more times.
7. Add 100 µL of FTN Enzyme Reagent into each well.
8. Cover the plate with a microplate sealer, and incubate the microplate at room temperature (20-28°C) for 30 minutes.
9. Wash Step (Can be performed manually or with automated washing):**Manual washing:** Carefully remove the incubation mixture by disposing the solution into a waste container. Fill each well with 350 µL working wash buffer and shake gently for 20-30 seconds. Discard the wash solution completely. Repeat 2 more times. After completing the last wash step, tap the plate on absorbent paper to remove residual liquid. **Automated washing:** Automatic plate washer must be calibrated to ensure efficient washing. Fill each well with 350 µL diluted wash buffer. Aspirate all wells completely. Repeat 2 more times.
10. Add 50 µL of TMB substrate A & 50 µL of TMB substrate B into each well.
11. Cover the microplate and incubate at room temperature (20-28°C) for 15 minutes.
12. Stop the reaction by adding 50 µL of Stop Solution to each well. Gently mix for 20 seconds. Add the Stop Solution in the same sequence as substrate addition. It is important to make sure that all the blue color changes completely to a yellow color.
13. Set the microplate reader wavelength at 450 nm. Measure the absorbance (OD) of each well within 30 minutes after adding Stop Solution. A filter of 610-640 nm can be used as a reference wavelength to optimize the assay result.

Flow chart of assay procedure

1.	Secure strips in microwell frame	Number of strips
2.	Add FTN Calibrators, Assay Control and specimens, respectively	25 µL
3.	Add FTN Biotin Reagent	100 µL
4.	Gently shake	10 seconds
5.	Incubate	20-28°C, 30 minutes
6.	Wash: manual or automatic	3 times
7.	Add FTN Enzyme Reagent	100 µL
8.	Cover and incubate	20-28°C, 30 minutes
9.	Wash: manual or automatic	3 times
10.	Add TMB substrate A & B and Gently rock	50 + 50 µL
11.	Cover and incubate	20-28°C, 15 minutes
12.	Add Stop Solution. Gently mix	50 µL 20 seconds
13.	Read result	450/610-640 nm within 30 minutes

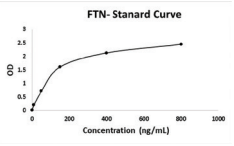
RESULTS CALCULATIONS

1. Calculate the mean absorbance value (A450/610-640) for each set of calibrators.
2. Construct a curve by plotting the mean value obtained for each calibrator against its concentration on graph paper with absorbance values on the vertical Y axis, and concentrations on the horizontal X axis.
3. Use the absorbance values for each specimen to determine the corresponding concentration of FTN in ng/mL from the curve.
4. Alternatively, if software is used, calculate the concentration of FTN following software menu.

INTERPRETATION AND QUALITY PARAMETERS

1. Results of a typical standard run are shown below:

Cal ID	Conc. (ng/mL)	OD
C1	0	0.009
C2	10	0.199
C3	50	0.706
C4	150	1.610
C5	400	2.127
C6	800	2.444
Patient ID	Conc. (ng/mL)	OD
1	61.45	0.810



2. The above data and figure are for example purposes and should not be used to calculate your result.
   If the OD value of a specimen is greater than that of the highest calibrator, it is recommended to dilute the specimen 1:5 and 1:10 with FTN '0' ng/mL calibrator, and retest.

EXPECTED NORMAL VALUE

A study of healthy, normal subjects, male and non-pregnant females (n=192) with the TRUSTwell Ferritin ELISA kit and established literature shows the concentrations of FTN in circulation listed below:

Expected Values for FTN (ng/mL)	
Males	10-220
Females	10-124

It is important to note that established values are guidelines only. Each lab should establish their own ranges for patients based on their own experience with the technology, methodology, local and populations. The values listed above should be used only as a guideline until the individual laboratory has established its own criteria.

PERFORMANCE CHARACTERISTICS

1) Analytical Sensitivity

OD values from 20 replicates of the '0' calibrator was run in a single assay on three lots of the TRUSTwell Ferritin ELISA and the mean and standard deviation (SD) were calculated. The analytical sensitivity of the test was interpolated from the dose response curve (DRC). The analytical sensitivity of the TRUSTwell Ferritin ELISA was determined to be 0.5 ng/mL at 2SD.

2) Analytical Specificity

Specificity of the TRUSTwell Ferritin ELISA kit was evaluated by spiking potential interfering substances into a human serum matrix. Specificity was calculated by deriving a ratio between the concentration of each tested metabolite and the concentration of FTN needed to displace the same amount of conjugate. There was no cross-reactivity observed at the concentrations listed below:

1.ASA	10 mg/mL	6. Hemoglobin	0.1 mg/mL
2.Bilirubin	10 µg/mL	7. Lipids	10 µg/mL
3.FSH	10,000 mIU/mL	8. LH	1,000 mIU/mL
4.hCG	100,000 mIU/mL	9. PRL	5,000 ng/mL
5. Biotin	60 ng/mL		

3) Accuracy

The accuracy of the TRUSTwell Ferritin ELISA system was determined by comparison with a commercial ELISA as a reference. Seventy-five (75) specimens with varying concentrations were tested with both TRUSTwell Ferritin ELISA and a reference ELISA by an external laboratory. The results are listed in the following table:

Method	Mean (ng/mL)	Least Square Regression	Corr. Coef. (r)
TRUSTwell (x)	189.73	y= 0.9762x – 0.091	0.997
Ref (y)	194.45		

4) Precision

(a) Intra-Assay Precision: Twenty replicates of each of three pooled human serum controls (low, middle, and high concentrations) were tested in the same assay. The mean, SD, and coefficient of variation (CV) were determined. The results are shown in the following table:

Sample	N	Mean (ng/mL)	SD	%CV
Low	20	59.07	4.8	9.3%
Middle	20	132.9	5.3	4.0%
High	20	399.7	31.3	7.8%

(b) Inter-Assay Precision: Three human serum pooled controls (low, middle, and high concentrations) were assayed in duplicates in 20 separate runs in 3 separate lots. The mean, SD, and CV were determined. The results are shown in the following table:

Sample	N	Mean (ng/mL)	SD	%CV
Low	20	51.8	4.8	9.3%
Middle	20	132.9	5.3	4.0%
High	20	399.7	31.3	7.8%

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to check assay performance. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

The following criteria should be met to consider assay results to be valid:

- The absorbance of '800' calibrator should be > 1.3 OD.
- CV's for duplicates of calibrators should be < 10%.
- FTN Assay Control should be within specified range on the vial label.

WARNING AND PRECAUTIONS

For *in vitro* Diagnostic Use

1. This instructions for use must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired kits.
3. Bring all reagents to room temperature (22-28°C) before use.
4. Do not use the components of any other type of test kit as a substitute for the components in this kit. Do not reuse the used microwells to test new samples.
5. Do not use serum derived from hemolyzed blood specimens for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mouth. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as biohazardous waste.
10. Prior to the first incubation and after adding Stop Solution, gently rock the microwells to ensure thorough mixing. Avoid the formation of air bubbles which results in inaccurate absorbance values. Avoid splashing liquid while rocking or shaking the wells.
11. Do not allow the microwells to dry between the end of the washing operation and the reagent distribution.
12. The enzyme-substrate reaction is very sensitive to metal ions. Thus, do not allow any metal elements to come into contact with the conjugate or TMB Substrate.
13. The enzyme-substrate is temperature dependent. Ensure that the room temperature for incubation falls between 22-28˚C.
14. The TMB substrate must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate must be stored in the dark.
15. Use a new dispensing tip for each specimen. Never use the specimen container to distribute conjugate and TMB Substrate. Always use new pipette tip for pipetting TMB substrate.
16. The wash procedure is critical. Wells must be aspirated completely before adding the Wash buffer or liquid reagents. Automatic washers must be validated with the test kit prior to use. Insufficient washing will result in poor precision and falsely elevated absorbance values.
17. Microplate reader must be calibrated per manufacturer's instruction to ensure accurate determination of absorbance. Non-calibrated reader often leads to invalid test results.
18. Avoid exposure to strong light during color development.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Results must be followed closely when testing the level of FTN in serum from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The test is limited to the quantitative determination of FTN concentration in human serum. Plasma samples may interfere with test results and should be avoided.
3. If the OD value of a specimen is greater than that of the highest calibrator, it is recommended to dilute the specimen and test again.
4. Any interpretation or use of this test result must also integrate other clinical findings as well as on the professional judgment of health care providers..

REFERENCES

1. de Benoist B et al., eds. Worldwide prevalence of anaemia 1993-2005. WHO Global Database on Anaemia Geneva, World Health Organization, 2008.
2. Garcia-Casal MN, Pasricha S, Martinez RX, Lopez-Perez L, Peña-Rosas J. Serum or plasma ferritin concentration as an index of iron deficiency and overload. Cochrane Database of Systematic Reviews 2015, Issue 7. Art. No.: CD011817.
3. WHO. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011.
4. WHO/UNICEF/UNU. Iron deficiency anaemia: assessment, prevention and control, a guide for programme managers. Geneva, World Health Organization, 2001

Index of Symbols

Consult instructions for use	REF Catalogue number	Use-by date
IVD For *in vitro* diagnostic use only	LOT Batch code	Tests per kit
Temperature limit 2-8°C	Do not re-use	Keep dry
Manufacturer	Date of manufacture	If device is non-sterile
Do not use if package is damaged	Keep away from sunlight	Warnings / Precautions

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