Human Ferritin (FE) ELISA

Cat No: K12-1703

Principle:
This is sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human FE in samples. Standards or Samples are added to the microtiter well which is pre-coated with Human FE monoclonal Antibody. Biotinylated Human FE antibodies are added to the microplate to form a complex. Subsequently Streptavidin-HRP conjugate is pipetted. After incubation and a washing step TMB Substrate A and B, are added. Blue color develops on incubation and the reaction is stopped with a Stop Solution to form a yellow color. The concentration of the Human FE in the samples is directly proportional to the yellow color developed in the wells.

Intended Use:
This Kit is used to assay the level of Human FE in human serum, plasma, saliva and other related tissue liquid. The Kit is For Laboratory / Research Use Only.

Materials provided in the kit:
1. Anti-Human FE Coated Microtitre Plate (96 wells) – 1 no
2. Biotinylated Human FE Antibody – 1 ml
3. Standard (concentrated, 480 ng/ml) – 0.5 ml
4. Streptavidin-HRP Conjugate – 6 ml
5. (30X) Wash Buffer – 20 ml
6. Standard Diluent – 3 ml
7. TMB Substrate A – 6 ml
8. TMB Substrate B – 6 ml
9. Stop Solution – 6 ml
10. Instruction Manual

Materials to be provided by the End-User:
1. Microplate Reader able to measure absorbance at 450 nm.
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.
9. Incubator

Storage Information:
1. All reagents should be stored at 2ºC to 8ºC.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 30 minutes prior before use, bring all components to room temperature (18-25ºC). Store all the components of the kit at its appropriate storage condition after use.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

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 samples 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 quantitation.
1. The kit cannot test samples which contain NaN_3, because NaN_3 inhibits HRP activity.
2. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.

3. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
4. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 20-min at the 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
5. **Saliva** -collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, if precipitation appeared, Centrifugal again. The Operation of Hydrothorax and cerebrospinal fluid Reference to it.
6. **Tissue samples-** After cutting the samples check the weight and add PBS (pH 7.2-7.4). Rapidly freeze with liquid nitrogen. Maintain samples at 2-8℃ after melting. Add PBS (pH 7.4). Homogenize by hand or grinder. Centrifuge for 20 mins at 2000-3000 rpm. Remove the supernatant.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Bring all reagents to Room Temperature prior to use.
2. To make 1X Wash Solution, add 10 ml of 30X Wash Buffer in 290 ml of DI water.

Procedural Notes:

1. After taking the kit out from 2-8˚C environment, the kit should be stabilized for 30 minutes at room temperature and then used. If the coated strips haven't been used up after opening, then the remaining strips should be stored in a sealed bag.
2. For each step, add sample with sample injector which should be calibrated frequently, in order to avoid unnecessary experimental tolerance.
3. The operation shall be carried out according to the instructions strictly. And test results must be based on the readings of the Microplate reader.
4. In order to avoid cross-contamination, it is forbidden to re-use the suction head and seal plate membrane in your hands.
5. All samples, washing buffer and each other component should be discarded according to infective material process.
6. Reagents not in used should be covered. Do not use reagent with different batches and use them before expiry date.
7. Substrate B is light-sensitive and so its prolonged exposure to light is forbidden.

Assay Procedure:

1) Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples should be run in duplicates or triplicates. A standard curve is required for each assay.
2) Standards Dilution: Prepare the standards as per the table given below using the provided standard Concentration and Standard Diluent.

Standard Concentration	Standard No	Dilution Particulars
480 ng/ml	---	500 ul of Original Standard
240 ng/ml	Standard No.5	120 ul Original Standard + 120 ul Standard diluent
120 ng/ml	Standard No.4	120 ul Standard No.5 + 120 ul Standard diluent
60 ng/ml	Standard No.3	120 ul Standard No.4 + 120 ul Standard diluent
30 ng/ml	Standard No.2	120 ul Standard No.3 + 120 ul Standard diluent
15 ng/ml	Standard No.1	120 ul Standard No.2 + 120 ul Standard diluent

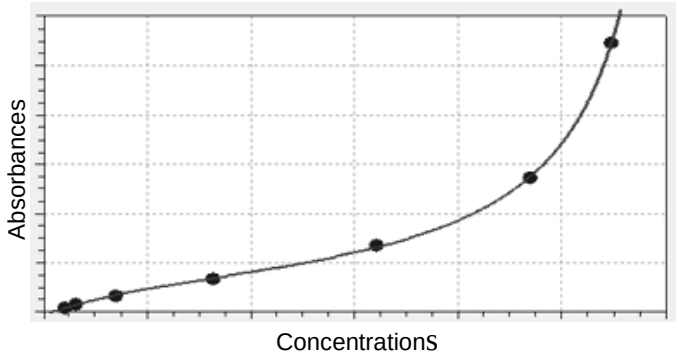
3) The quantity of the plates depends on the quantities of samples and standards to be tested. It is suggested to remove the number of strips required for the assay.
4) Pipette **50 ul** of **Standards** and **40 ul Samples** into the respective wells as mentioned in the work list.
Note do not add the sample, Biotin Conjugate and Streptavidin-HRP to the blank well.
5) Pipette **10 ul** of **Biotinylated Human FE Antibody** into each sample well.
Do not pipette into the blank and standards wells.
6) Pipette **50 ul** of **HRP Conjugate** into each sample and standards well.
Do not pipette into the Blank well.
7) Cover the plate and incubate for 1 hour at 37°C in the incubator.
8) Aspirate and wash plate 4 times with **1X Wash Buffer** and blot residual buffer by firmly tapping the plate on an absorbent paper. Wipe off any liquid from the bottom of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
9) Then add **TMB Substrate A 50 ul**, then **TMB Substrate B 50 ul** to each well including Blank well. Gently mixed, incubate for 10 min at 37°C in dark.
10) Pipette **50 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

11) Read the absorbance at 450 nm within 15 minutes after adding the Stop Solution. Blanking the zero standard for net absorbance.

Calculation of Results:
Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using standard graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown concentrations, find the unknown's 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 concentration. If samples were diluted, multiply by the appropriate dilution factor.

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.

Typical Graph



Precautions:
Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

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 **1.446 ng/ml.**

Specificity:
The antibodies used in the kit for capture and detection are specific for human Ferritin.

Assay Range:
15 ng/ml to 240 ng/ml.

Precision:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Linearity:
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of CAMK2B and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
serum (n=5)	84-107%	87-108%	82-112%
EDTA plasma (n=5)	83-102%	83-115%	83-118%
heparin plasma (n=5)	83-99%	80-95%	82-93%

LIMITED WARRANTY

KinesisDx 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 KinesisDx, or against damages resulting from such non-KinesisDx made products or components. KinesisDx 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 KinsisDx.

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Troubleshooting:

Problem	Possible cause	Investigation/Actions
High Absorbances	1. Cross-contamination from other specimens	> Repeat assay taking care when washing and pipetting.
	2. Insufficient or inefficient washing or reading	> Check washer efficiency
	3. Wavelength of filter not correct.	> Check that the wavelength is 450nm. If a dual wavelength spectrophotometer is available, set the reference filter between 600-650 nm.
	4. High assay background.	> Repeat assay and include a well that contains only sample diluent or sample absorbent (i.e. a blank well).
	5. Contaminated TMB	> Check that TMB is colorless or faint blue.
	6. Incubation time too long or incubation temperature too high.	> Check incubation time and temperature. > Check incubator is at the correct temperature.
	7. Incorrect dilution of serum	> Repeat assay, ensuring correct serum dilution is used.
Low Absorbances	1. Incubation time too shot or incubation temperature too low.	> Ensure time and temperature of assay incubation are correct. > Check incubator is set at the correct temperature.
	2. Incorrect dilution or pipetting of sera	> Repeat assay ensuring correct dilutions and volumes are used. > Ensure controls are sufficiently mixed.
	3. Incorrect filter wavelength.	> Check the wavelength is set at 450nm. If a dual wavelength spectrophotometer is available, set the reference filter between 600-650nm.
	4. Contaminated Conjugate solution.	> Dispense conjugate directly from the bottle using clean pipette tip; avoid transferring Conjugate to another container if possible. > Do not return unused Conjugate to bottle. > Ensure all pipettes and probes used to dispense the Conjugates are clean and free from serum, detergent and bleach.
	5. Kit has expired.	> Check expiration date of kit and do not use if expired.
	6. Air blank reading high.	Investigate causes of high background absorbance.
	7. Incorrect storage of kit.	> Ensure kit is stored at 2-8ºC,plate is sealed in foil pouch and desiccant sachet is blue/purple.
	8. Kit reagents not equilibrated at room temperature	> Allow sufficient time for reagents to equilibrate to room temperature prior to assay.
	9. Incorrect reagents used.	> Check the reagents used match those listed on the specification sheet.
	10. Over washing of plate (e.g. inclusion of a long soak step).	> Repeat assay using recommended wash procedure.
Poor Duplicates	1. Poor mixing of samples.	> Mix reagents gently and equilibrate to room temperature.
	2. Poor pipette precision	> Calibration may need to be checked. > Check pupating technique-change pipette tip for each sample and ensure excess liquid is wiped from the outside of the tip.
	3. Addition of reagents at inconstant timing intervals; reagent addition takes too long, air bubbles when adding reagents.	> Use consistent timing when adding reagents. > Ensure all dilutions are made before commencing addition to plate. > Improve pipetting technique and skill.
	4. Inefficient washing - Wash buffer left in wells, inconsistent washing, inadequate washing.	> Tap out wash buffer after washing. > Check wells are sufficiently and uniformly filled and aspirated when washing.
	5. Reader not calibrated or warmed up prior to plate reading.	> Check reader precision > Check reader manual to ascertain warm up time of instrument.
	6. Optical pathway not clean	> Gently wipe bottom of plate. > Check reader light source and detector are clean.
	7. Spillage of liquid from wells	> Repeat assay, taking care not to knock the plate or splash liquid
	8. Serum samples exhibit microbial growth, haemolysis or lipaemia.	> It is not recommended to use serum samples exhibiting microbial growth, haemolysis or lipaemia.
	9. Uneven well volumes due to evaporation.	> Cover plate with a lid or plate sealer (not provided).
All wells yellow	1. Contaminated TMB.	> Check TMB is colorless or faint blue.
	2. Contaminated reagents (e.g. Conjugate, Wash buffer).	> Check reagents for turbidity.
	3. Incorrect dilution of serum.	> Repeat assay, ensuring correct serum dilution is used.
	4. Incorrect storage of kit.	> Ensure kit is stored at 2-8ºC, plate is sealed in foil pouch and desiccant sachet is blue / purple.
	5. Inefficient washing- Wash buffer left in wells, inconsistent washing, inadequate washing.	> Tap out wash buffer after washing. > Check wells are sufficiently and uniformly filled an aspirated when washing.
	6. If Conjugate reconstitute is required – Conjugate reconstituted incorrectly.	> Repeat assay ensuring Conjugate is reconstituted according to assay method.

All wells negative	1. Test not performed correctly – correct reagents not added or not added in the correct sequence.	> Check procedure and check for unused reagents. > Ensure that Stop Solution was not added before Conjugate or TMB. > Ensure that serum was diluted in correct Sample diluent; e.g. do not use Sample Absorbent for an IgG ELISA.
	2. Contaminated Conjugate solution.	> Dispense Conjugate directly from the bottle using a clean pipette tip; avoid transferring Conjugate to another container if possible. > Do not return unused Conjugate to bottle. > Ensure all pipettes and probes used to dispense the Conjugate are clean and free from serum, detergent and bleach.
	3. Over- washing of plate (e.g. inclusion of a long soak step).	> Repeat assay using recommended wash procedure.
	4. Incorrect storage of kit.	> Ensure kit is stored at 2-8ºC, plate is sealed in foil pouch and desiccant sachet is blue / purple.
	5. Wash Buffer made up with Stop Solution instead of Wash Buffer Concentrate	> Ensure Wash Buffer is made up correctly.