Human Free Fatty Acids, FFA GENLISA™ ELISA

REF : KBH2013

Ver3.0

RUO

Immunoassay for Quantitative Determination of Human Free fatty Acids, FFA levels in serum, plasma, and other biological samples.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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Introduction:

The GENLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Intended Use:

The Human Free fatty Acids, FFA GENLISA™ ELISA kit is used as an analytical tool for quantitative determination of Human Free fatty Acids, FFA in serum, plasma, and other biological samples.

Principle:

The method employs sandwich ELISA technique. Human Free fatty Acids, FFA monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Human Free fatty Acids, FFA present in the sample are bound by the antibodies. Anti FFA: HRP Conjugate is added. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Human Free fatty Acids, FFA in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Human Free fatty Acids, FFA antibody Coated Microtiter plate – 8 X 12 wells
2. Standard, Human Free Fatty Acids, FFA (concentrated, 540 nmol/ml) – 0.5 ml
3. Standard Diluent – 1.5 ml
4. Anti-FFA:HRP Conjugate – 6 ml
5. Sample diluent – 6 ml
6. (20X) Wash Buffer– 25 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. 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 pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
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.
2. For Research Use Only.

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, re-centrifuge.
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, re-centrifuge.
5. **Urine samples -** Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuged again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.
6. **Cell samples -** If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuged again.
7. **Tissue samples -** Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80℃ for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4℃. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Note:

1. Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay cannot be performed immediately, samples can be stored at -20℃ Repeated freeze-thaw cycles should be avoided.
2. Our kits cannot be used for samples with NaN3 which can inhibit the activity of HRP.

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

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X);** dilute **25 ml of (20X) Wash Buffer in 475 ml of DI water.**
4. **Sample Preparation:** Add 10ul of sample and to this add 40ul of sample diluent, mix well with gently shaking. Samples should be loaded onto the bottom without touching the well wall
5. **Standards Preparation**: Dilute 300 ul of original **Standard (540 nmol/ml)** with 150 ul of standard diluent to generate a **360 nmol/ml Standard stock solution**. Keep the standard for 15 mins with gentle agitation before making further dilutions. Prepare the **Standards** by serially diluting the standard stock solution as per the below table

Standard Concentration	Standard Vial	Dilution Particulars
540 nmol/ml	Original Standard	Original Standard provided in the Kit
360 nmol/ml	Standard No.5	300 ul Original Standard + 150 ul Standard diluents
240 nmol/ml	Standard No.4	300 ul Standard No.4 + 150 ul Standard Diluent
120 nmol/ml	Standard No.3	150 ul Standard No.3 + 150 ul Standard Diluent
60 nmol/ml	Standard No.2	150 ul Standard No.2 + 150 ul Standard Diluent
30 nmol/ml	Standard No.1	150 ul Standard No.5 + 150 ul Standard Diluent
0 nmol/ml	Standard No.0	150 ul Standard Diluent

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Human Free fatty Acids, FFA. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Free fatty Acids, FFA present in the sample.
3. Human Free fatty Acids, FFA concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Human Free fatty Acids, FFA.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
   Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add **50 ul prepared Standards and diluted sample** to respective standard wells.

3. Cover the plate with a sealer and incubate for **30 minutes** at **37°C.**

4. 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.

5. Add **50 ul of Anti-FFA:HRP Conjugate** into each well except the blank control well.

6. Cover the plate with a sealer and incubate for **30 minutes** at **37°C.**

7. 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.

8. Add **100 ul of TMB Substrate** in each well.

9. Incubate the plate at **37°C** for **15 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

10. Pipette out **100 ul of Stop Solution**. Wells should turn from blue to yellow in color.

11. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using 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 Human Free fatty Acids, FFA 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 Human Free fatty Acids, FFA Concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4-PL 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.

Performance Characteristics of the Kit:
This kit has been validated. Please view the details herein below.

Standard Calibration Range:
30 nmol/ml - 360 nmol/ml

Sensitivity:
Limit Of Quantification:
It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 1.5 nmol/ml

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

Safety Precautions:

- **This kit is For Research 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.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Zero Std Zero Std			
1B 2B	Standard No.1 Standard No.1			
1C 2C	Standard No.2 Standard No.2			
1D 2D	Standard No.3 Standard No.3			
1E 2E	Standard No.4 Standard No.4			
1F 2F	Standard No.5 Standard No.5			
1G 2G	Sample			
1H 2H	Sample			
3A 4A	Sample			
3B 4B	Sample			

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THANK YOU FOR USING KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	Coated Microtiter Plate (8x12 wells)
STD	Standard
HRP CONJ	Anti-FFA:HRP Conjugate
STD DIL	Standard Diluent
SAMP DIL	Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature