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Human FGF-Basic GENLISA™ ELISA

REF : KB1040

Ver.2.1

RUO

Enzyme Immunoassay for the Quantitative Determination of Human FGF-Basic in human 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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Human FGF-Basic GENLISA™ ELISA

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.

Long Name: Fibroblast Growth Factor basic

Entrez Gene IDs: 2247 (Human); 14173 (Mouse); 281161 (Bovine); 403857 (Canine); 100033955 (Equine)

Alternate Names: Basic fibroblast growth factor bFGF; Basic fibroblast growth factor; bFGF; FGF basic; FGF2; FGF-2; FGFBprostatropin; fibroblast growth factor 2 (basic); HBGF-2; heparin-binding growth factor 2; Prostatropin

Intended Use:

The Human FGF-Basic GENLISA™ ELISA kit is used as an analytical tool for quantitative determination of Human FGF-Basic in serum, plasma and other biological samples.

Principle:

FGF basic/FGF2/bFGF is a growth factor that functions in angiogenesis, wound healing, tissue repair, learning and memory, and the morphogenesis of heart, bone, and brain. It is upregulated in response to inflammatory stimuli and in many tumors. FGF basic/FGF2/bFGF binds to FGFR1c and 2c. Its bioactivity is modulated by a number of other binding partners including heparin, Integrin alpha V beta 3, soluble FGFR1, FGF-BP, free gangliosides, Thrombospondin, Pentraxin 3/TSG-14, Fibrinogen, alpha 2-Macroglobulin, PDGF, and CXCL4/PF4. These molecules act as cellular coreceptors or adhesion partners, extracellular matrix decoys or reservoirs, and soluble scavengers or chaperones. In particular, the interaction of FGF basic/FGF2/bFGF with cell surface heparan sulfate proteoglycans (HSPG) is required for the binding and activation of FGF receptors.

Materials Provided:

1. Microtiter Coated Plate (12 x 8 wells) – 1 no
2. Recombinant Human FGF-Basic Standard (lyophilized, concentrated, 280 ng/ml) – 2 vials
3. Human FGF-Basic Biotin Conjugated Detection Antibody ((lyophilized, concentrated, 6 ug/ml) – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial
5. (20X) Wash Buffer – 25 ml
6. (1X) Assay Diluent – 50 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. 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. Semi log graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:

1. Store main kit components at 2-8°C.
2. Store recombinant Standard and Detection Antibody at 2-8°C. Upon reconstitution, aliquot recombinant protein and detection antibody into polypropylene vials and store at -20°C as per assay requirements.
3. 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 quantitation.
Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at temperature < -20°C. Avoid repeated freeze/thaw cycles.

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.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

Reagent Preparation:

Please refer to lot specific instructions for preparation of the reagents.

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. **Standards Preparation:** Reconstitute the lyophilized vial with 10ul of Assay Diluent (1X) to generate a 280 ng/ml. Dilute 3.57 ul of original reconstituted Standard (280 ng/ml) with 996.43 ul of Assay Diluent (1X) to generate a 1000 pg/ml top standard. Perform serial dilutions by using top 1000 pg/ml top standard as per the below table. Thus, the Human FGF basic standard concentrations are 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml and 15.6 pg/ml. Assay Diluent (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
280 ng/ml	Standard, lyophilized	Lyophilized Standard provided in the Kit + 10ul Assay Diluent (1X)
1000 pg/ml	Standard No.7	3.57 ul Original Standard + 996.43 ul Assay Diluent (1X)
500 pg/ml	Standard No.6	500ul Standard No.7 + 500ul Assay Diluent (1X)
250 pg/ml	Standard No.5	500ul Standard No.6 + 500ul Assay Diluent (1X)
125 pg/ml	Standard No.4	500ul Standard No.5 + 500ul Assay Diluent (1X)
62.5 pg/ml	Standard No.3	500ul Standard No.4 + 500ul Assay Diluent (1X)
31.25 pg/ml	Standard No.2	500ul Standard No.3 + 500ul Assay Diluent (1X)
15.6 pg/ml	Standard No.1	500ul Standard No.2 + 500ul Assay Diluent (1X)
0 pg/ml	Standard No.0	500ul Assay Diluent (1X)

3. Add **100 ul** of **Standards** and **Samples** to respective wells.

4. Seal plate and incubate at Room Temperature for 2 hours.

5. Aspirate and wash plate 4 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 prepared **FGF-Basic Biotin Conjugated Antibody** to all the wells.

7. Seal plate and incubate at Room Temperature for 2 hours.

8. Wash plate 4 times with **Wash Buffer (1X)** as in step 5.

9. Add **100 ul** of diluted **Streptavidin-HRP** solution to each well, seal plate and incubate for 30 minutes at Room Temperature.

10. Wash plate 4 times with **Wash Buffer (1X)** as in step 5.

11. Add **100 ul** of **TMB Substrate** solution and incubate in the dark for 30 minutes at Room Temperature. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

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

13. Read Absorbance at 450 nm within 30 minutes of stopping reaction.

Calculation of Results:

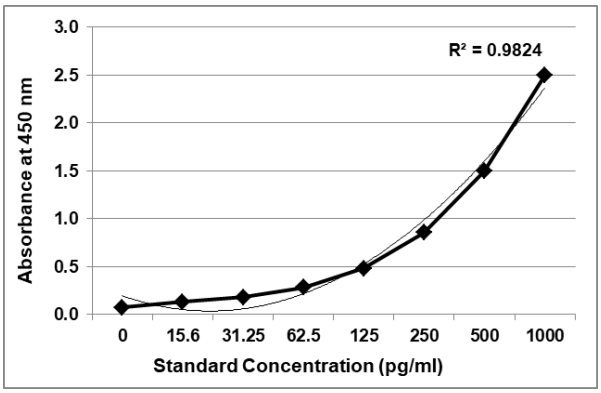
Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Human FGF-Basic 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 unknown Human FGF-Basic concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4PL (2nd order) is best recommended for automated results.

Typical Data

Standard Concentration (pg/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.074	0.073	0.074	--	--
15.6	0.134	0.130	0.132	16.6	106.5
31.25	0.176	0.182	0.179	30.4	97.4
62.5	0.276	0.292	0.284	62.0	99.3
125	0.480	0.488	0.484	124.8	99.8
250	0.841	0.873	0.857	251.1	100.4
500	1.442	1.549	1.495	499.2	99.8
1000	2.453	2.554	2.503	1000.2	100.0

Typical Graph



Abs = absorbance at 450nm

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 **15 pg/ml.**

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Human FGF-Basic ELISA.

Cross Reactivity:
This assay recognizes natural and recombinant human IL-6. The markers listed below were prepared at 50 pg/ml in Assay Diluent and assayed for cross-reactivity. No significant cross-reactivity or interference was observed.

Recombinant human:
FGF acidic FGF-4 FGF-6 KGF/FGF-7

This kit shows cross reactivity for:
- A sample containing 250 pg/mL of recombinant mouse FGF basic reads as 75 pg/mL (30% cross-reactivity).
- A sample containing 50 ng/mL of bovine FGF acidic reads as 96 pg/mL (0.20% cross-reactivity).
- A sample containing 1.6 ng/mL of bovine FGF basic reads as 2200 pg/mL (140% cross-reactivity).

Assay Range:
15.6 pg/ml to 1000 pg/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 Human FGF Basic 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%

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.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

Components NOW (2-8ºC) → 30 mins → RT Before Use → 

→ Thaw at Room Temperature (18-24ºC) → USE

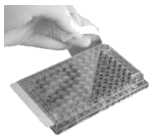
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components RESULTS → **COOL (2-8ºC)** → **THAW (22±4ºC)** → NO CHANGE IN

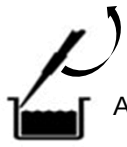
AVOID **THAW (22±4ºC)** **COOL (2-8ºC)** **AVOID**

3.  Pipette **100 ul Standards** into respective Standard wells.

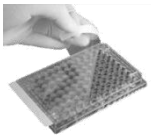
4.  Pipette **100 ul Samples** into the sample wells.

5. Cover plate  and incubate for 

at room temperature.

6.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

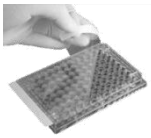
7.  Pipette **100 ul** diluted **Biotin Conjugated Antibody** to all wells.

8. Cover plate  and incubate for 

at room temperature.

9.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

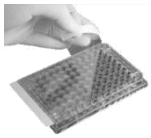
10.  Pipette **100 ul** of diluted **Streptavidin:HRP** to all wells

11. Cover plate  and incubate for 

at room temperature

12.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

13.  Pipette **100 ul TMB Substrate** into each wells

14. Cover plate  and incubate **for** 

at room temperature.

15.  Pipette **100 ul Stop Solution** into each well.

16. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

LIMITED WARRANTY

Krishgen Biosystems 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 Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems 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 Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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SYMBOLS KEY

MTP	Human FGF Basic antibody coated microtiter plate (12x8 wells)
STD	Recombinant Human FGF-Basic Standard
BIO CONJ	Biotin Conjugated Detection Antibody
STRP HRP	Streptavidin Horseradish Peroxidase
1X ASY DIL	(1X) Assay 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