

GENLISA® Human Hypoxia Inducible Factor 1 Alpha, HIF-1alpha ELISA

REF: KBH1860

Ver 1.0

Pack Size: 96 wells

For Research Use Only

Enzyme Immunoassay for the Quantitative Determination of Hypoxia Inducible Factor 1 Alpha, HIF-1alpha 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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REF KBH1860

 96 tests

Krishgen Biosystems Private Limited

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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 GENLISA® Human Hypoxia Inducible Factor 1 Alpha, HIF-1alpha ELISA kit is used as an analytical tool for quantitative determination of Human Hypoxia Inducible Factor 1 Alpha, HIF-1alpha in serum, plasma and other biological samples.

Principle:

The method employs sandwich ELISA technique. Human Hypoxia Inducible Factor 1 Alpha, HIF-1alpha antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Human Hypoxia Inducible Factor 1 Alpha, HIF-1alpha present in the sample are bound by the antibodies. Biotin labeled antibody is added and followed by Streptavidin:HRP is pipetted and incubated to form a complex. 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 Hypoxia Inducible Factor 1 Alpha, HIF-1alpha in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Human HIF-1alpha Antibody Coated Microtiter Plate (12 x 8 wells) – 1 no
2. Standard, Human HIF-1alpha (lyophilized, concentrated, 4000 pg/ml) – 2 vials
3. Biotinylated HIF-1alpha Antibody (concentrated) - 120 ul
4. Streptavidin:HRP Conjugate (concentrated) - 120 ul
5. Standard/Sample Diluent - 20 ml
6. Biotin Antibody Dilution Buffer - 13 ml
7. HRP Conjugate Dilution Buffer - 13 ml
8. (25X) Wash Buffer – 30 ml
9. TMB Substrate – 10 ml
10. Stop Solution – 10 ml
11. 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. Reagents like Microtiter plate, Standard, Biotin and HRP should be stored at -20°C for stability.
2. Reagents like Diluents, Wash buffer, TMB substrate and Stop solution should be stored at 2°C-8°C for stability.
3. All the reagents and wash solutions are stable until the expiration date of the kit.
4. Before using, bring all components to room temperature (18-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
5. 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.



Sample Preparation and Storage:

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

1. 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.
2. **Serum**- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma**- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Tissue Samples**- Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH 7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.
5. **Cell Culture Supernatant**- Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.

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

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 **30 ml of 25X Wash Buffer in 720 ml of DI water**.
4. **Biotinylated HIF-1alpha Antibody Working Solution**: Calculate required total volume of the working solution: 0.1ml / well x quantity of wells. Allow 0.1-0.2 ml more than the total volume. Dilute the Biotinylated HIF-1alpha Antibody (concentrated) with Biotin Antibody Dilution Buffer at 1:100 and mix them thoroughly (i.e. Add 1 ul Biotinylated HIF-1alpha Antibody into 99 ul Biotin Antibody Dilution Buffer.)
5. **Streptavidin:HRP Conjugate Working Solution**: Calculate required total volume of the working solution: 0.1ml / well x quantity of wells. Allow 0.1-0.2 ml more than the total volume. Dilute the Streptavidin:HRP Conjugate with Streptavidin:HRP Conjugate Dilution Buffer at 1:100 and mix them thoroughly (i.e. Add 1 ul of Streptavidin:HRP Conjugate into 99 ul of Streptavidin:HRP Conjugate Dilution Buffer).
6. **Standards Preparation**: Reconstitute original Hypoxia Inducible Factor 1 Alpha, HIF-1alpha with 1.0 ml of Standard/Sample Diluent. Keep the standard for 10 mins with gentle agitation before making further dilutions. Prepare the additional Standards by serially diluting the standard stock solution as per the below table.

Standard Concentration	Standard Vial	Dilution Particulars
4000 pg/ml	Standard No.7	Reconstitute with 1.0 ml Standard/Sample Diluent
2000 pg/ml	Standard No.6	250 ul Standard No.7 + 250 ul Standard/Sample Diluent
1000 pg/ml	Standard No.5	250 ul Standard No.6 + 250 ul Standard/Sample Diluent
500 pg/ml	Standard No.4	250 ul Standard No.5 + 250 ul Standard/Sample Diluent
250 pg/ml	Standard No.3	250 ul Standard No.4 + 250 ul Standard/Sample Diluent
125 pg/ml	Standard No.2	250 ul Standard No.3 + 250 ul Standard/Sample Diluent
62.5 pg/ml	Standard No.1	250 ul Standard No.2 + 250 ul Standard/Sample Diluent
0 pg/ml	Standard No.0	250 ul Standard/Sample Diluent only

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 Hypoxia Inducible Factor 1 Alpha, HIF-1alpha. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Hypoxia Inducible Factor 1 Alpha, HIF-1alpha present in the sample.
3. Human Hypoxia Inducible Factor 1 Alpha, HIF-1alpha 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 Hypoxia Inducible Factor 1 Alpha, HIF-1alpha.
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 compromise of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. 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 **100 ul prepared Standards, blank and Samples** to respective wells.
3. Cover the plate with a sealer and incubate for **90 minutes** at **37°C**.
4. Aspirate and wash plate 3 times with diluted 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. Pipette **100 ul Biotinylated HIF-1alpha Antibody Working Solution** to all wells.
6. Cover the plate with a sealer and incubate for 60 minutes at 37°C.
7. Aspirate and wash as per Step (4) above.
8. Pipette **100 ul Streptavidin:HRP Conjugate Working Solution** to all wells.
9. Cover the plate with a sealer and incubate for **30 minutes** at **37°C**.
10. Repeat the wash step as per Step (4) above.
11. Pipette **100 ul TMB Substrate** to all the wells.
12. 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.
13. Pipette **50 ul of Stop Solution** to all wells. The wells should turn from blue to yellow in color.
14. 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 Hypoxia Inducible Factor 1 Alpha, HIF-1alpha 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 Hypoxia Inducible Factor 1 Alpha, HIF-1alpha 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.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:

This kit has been validated. Please view the details herein below.

Standard Calibration Range:

62.5 pg/ml – 4000 pg/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 37.5 pg/ml.

Specificity:

This assay has high sensitivity and excellent specificity for detection of Hypoxia Inducible Factor 1 Alpha, HIF-1alpha. No significant cross-reactivity or interference between Hypoxia Inducible Factor 1 Alpha, HIF-1alpha and analogues was observed.

Recovery:

Matrices listed below were spiked with certain level of Hypoxia Inducible Factor 1 Alpha, HIF-1alpha and the recovery rates were calculated by comparing the measured value to the expected amount of Hypoxia Inducible Factor 1 Alpha, HIF-1alpha in samples.

Matrix	Recovery Range (%)	Average (%)
serum(n=5)	92-106	99
EDTA plasma(n=5)	80-95	86
cell culture media(n=5)	88-103	95

Linearity:

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Hypoxia Inducible Factor 1 Alpha, HIF-1alpha and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	87-96%	92-105%	89-102%	92-101%
EDTA plasma(n=5)	80-93%	80-93%	89-97%	85-94%
cell culture media(n=5)	87-101%	86-94%	85-95%	89-99%

Precision:

Intra-Assay: CV<10%

Inter-Assay: CV<10%

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.

- 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 Ratand 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	Blank Blank			
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	Standard No.6 Standard No.6			
1H 2H	Standard No.7 Standard No.7			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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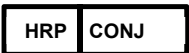
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SYMBOLS KEY

	Coated Microtiter Plate (12x8 wells)
	Standard
	Biotinylated Antibody
	Conjugate Horseradish Peroxidase
	Biotin Antibody Dilution Buffer
	HRP Conjugate Dilution Buffer
	Standard/Sample Diluent
	(25X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature