Human Homeobox Protein Nkx-2.5, NKX2-5 GENLISA™ ELISA

REF : KBH5433

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Determination of Human Homeobox Protein Nkx-2.5, NKX2-5 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 KBH5433 Σ **96 tests**

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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 Homeobox Protein Nkx-2.5, NKX2-5 GENLISA™ ELISA kit is used as an analytical tool for quantitative determination of Human Homeobox Protein Nkx-2.5, NKX2-5 in serum, plasma and other biological samples.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Human Homeobox Protein Nkx-2.5, NKX2-5 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 Homeobox Protein Nkx-2.5, NKX2-5 in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

- Anti-Human Homeobox Protein Nkx-2.5, NKX2-5 Antibody Coated Microtiter Plate (8 x 12 wells) - 1 no
- Human Homeobox Protein Nkx-2.5, NKX2-5 Standard (lyophilized, concentrated, for actual concentration please refer vial label) - 2 vials
- Biotinylated Human Homeobox Protein Nkx-2.5, NKX2-5 Antibody (concentrated) - 120 ul
- Streptavidin:HRP Conjugate (concentrated) - 120 ul
- Sample Diluent - 20 ml
- Biotin Antibody Dilution Buffer - 10 ml
- HRP Conjugate Dilution Buffer - 10 ml
- (25X) Wash Buffer - 30 ml
- TMB Substrate - 10 ml
- Stop Solution - 10 ml
- Instruction Manual

Materials to be provided by the End-User:

- Microtiter Plate Reader able to measure absorbance at 450 nm.
- Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
- Deionized (DI) water
- Wash bottle or automated microplate washer
- Clean tubes and Eppendorf tubes
- Precision single and multi-channel pipette and disposable tips.
- 37°C incubator
- Timer.

Handling/Storage:

- All reagents should be stored as indicated on the component label.
- All the reagents and wash solutions should be used within 12 months from manufacturing date.
- 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.
- The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

- Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
- 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.

- 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.
- **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
- **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.
- **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
- **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.
- **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 (pH7.4) 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.

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

Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples contain very high concentrations of the analyte, dilute the samples with PBS first and then do further dilutions of the samples with the provided Sample Diluent in the kit.

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

- Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
- Bring all reagents to Room temperature before use.
- To make **Wash Buffer (1X);** dilute **30 ml of (25X) Wash Buffer in 720 ml of DI water**.
- **Biotinylated Human Homeobox Protein Nkx-2.5, NKX2-5 Antibody Working Solution**: Prepare it within 1 hour before experiment. Calculate required total volume of the working solution: 0.1 ml / well x quantity of wells. (Allow 0.1-0.2 ml more than the total volume. Dilute the Biotinylated Human Homeobox Protein Nkx-2.5, NKX2-5 Antibody (concentrated) with Biotin Antibody Dilution Buffer at 1:100 and mix them thoroughly (i.e. Add 1 ul Biotinylated Human Homeobox Protein Nkx-2.5, NKX2-5 Antibody into 99 ul Biotin Antibody Dilution Buffer).
- **Streptavidin:HRP Conjugate Working Solution**: Prepare it within 30 minutes before experiment. 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).
- **Standards Preparation**: Reconstitute original Human Homeobox Protein Nkx-2.5, NKX2-5 Standard with 1 ml of Sample Diluent. Keep the standard for 15 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
as per label	original standard provided	Reconstitute with 1 ml Sample Diluent
1:1	Standard No.8	300 ul (reconstituted) Original Standard + 300 ul Sample Diluent
1:1	Standard No.7	300 ul Standard No.8 + 300 ul Sample Diluent
1:1	Standard No.6	300 ul Standard No.7 + 300 ul Sample Diluent
1:1	Standard No.5	300 ul Standard No.6 + 300 ul Sample Diluent
1:1	Standard No.4	300 ul Standard No.5 + 300 ul Sample Diluent
1:1	Standard No.3	300 ul Standard No.4 + 300 ul Sample Diluent
1:1	Standard No.2	300 ul Standard No.3 + 300 ul Sample Diluent
0 pg/ml	Standard No.1	300 ul Sample Diluent only

Note: actual concentration for the 1:1 dilution is to be determined as per the concentration indicvated on the standard vial label provided with the kit

Procedural Notes:

- In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
- High Dose Hook Effect may be observed in samples with very high concentrations of Human Homeobox Protein Nkx-2.5, NKX2-5. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Homeobox Protein Nkx-2.5, NKX2-5 present in the sample.
- Human Homeobox Protein Nkx-2.5, NKX2-5 concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
- 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 Homeobox Protein Nkx-2.5, NKX2-5.
- It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
- Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
- 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.
- 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:

- It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.
- Add **100 ul prepared Standards and diluted Samples** to respective wells.
- Cover the plate with a sealer and incubate for 90 minutes at 37°C.
- Aspirate and wash plate 4 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.
- Pipette **100 ul Biotinylated Human Homeobox Protein Nkx-2.5, NKX2-5 Antibody Working Solution** to all wells.
- Cover the plate with a sealer and incubate for 60 minutes at 37°C.
- Aspirate and wash as per Step (4) above.
- Pipette **100 ul Streptavidin:HRP Conjugate Working Solution** to all wells. Mix well.
- Cover the plate with a sealer and incubate for 30 minutes at 37°C.

- Aspirate and wash as per Step (4) above.

- Pipette **90 ul TMB Substrate** in all the wells.
- Incubate the plate at **37°C** for **10 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
- Pipette **50 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.
- 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 Homeobox Protein Nkx-2.5, NKX2-5 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 Homeobox Protein Nkx-2.5, NKX2-5.

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

Specificity:
This assay has high sensitivity and excellent specificity for detection of Human Homeobox Protein Nkx-2.5, NKX2-5. No significant cross-reactivity or interference between Human Homeobox Protein Nkx-2.5, NKX2-5 and analogues was observed.

Recovery
Matrices listed below were spiked with certain level of Human Homeobox Protein Nkx-2.5, NKX2-5 and the recovery rates were calculated by comparing the measured value to the expected amount of Human Homeobox Protein Nkx-2.5, NKX2-5 in samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	85-101	93
EDTA Plasma(n=5)	85-105	95
Heparin Plasma(n=5)	87-104	97

Precision:
Intra-Assay: CV<8%
Inter-Assay: CV<10%

Linearity
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Human Homeobox Protein Nkx-2.5, NKX2-5 and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Serum(n=5)	85-101%	86-101%	93-102%
EDTA Plasma(n=5)	84-95%	84-98%	82-98%
Heparin Plasma(n=5)	91-98%	81-100%	82-98%

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	Standard No.1 Standard No.1			
1B 2B	Standard No.2 Standard No.2			
1C 2C	Standard No.3 Standard No.3			
1D 2D	Standard No.4 Standard No.4			
1E 2E	Standard No.5 Standard No.5			
1F 2F	Standard No.6 Standard No.6			
1G 2G	Standard No.7 Standard No.7			
1H 2H	Standard No.8 Standard No.8			
3A 4A	Sample			
3B 4B	Sample			

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SYMBOLS KEY

MTP	Coated Microtiter Plate (8x12 wells)
STD	Standard
BIOTIN AB	Biotinylated Antibody
HRP CONJ	Conjugate Horseradish Peroxidase
BIOTIN DIL	Biotin Antibody Dilution Buffer
HRP DIL	HRP Antibody Dilution Buffer
SAMP DIL	Sample Diluent
25X WASH BUF	(25X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature