






# Human NADPH Oxidase 5, NOX5 GENLISA™ ELISA

**REF** : KBH5738

Ver 2.0

**RUO**

Enzyme Immunoassay for the Quantitative Determination of NADPH oxidase 5, NOX5 in human serum, plasma and other biological samples.

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

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**REF** KBH5738

 96 tests

**KRISHGEN BioSystems** For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005  
For Asia/India Customers: +91(22)-49198700  
Email: sales@krishgen.com | http://www.krishgen.com

**Introduction:**

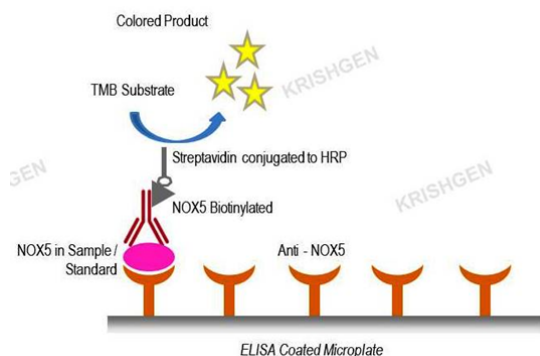
The GENLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional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

**Intended Use:**

The Human NADPH oxidase 5, NOX5 GENLISA™ ELISA kit is used as an analytical tool for quantitative determination of Human NADPH oxidase 5, NOX5 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 NADPH oxidase 5, NOX5 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 NADPH oxidase 5, NOX5 in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

**Materials Provided:**

1. Human NOX5 Antibody Coated Microtiter Plate (8 x 12 wells) - 1 no
2. Human NOX5 Standard (lyophilized, concentrated, 1600 pg/ml) - 2 vials
3. Biotinylated NOX5 Antibody (concentrated) - 120 ul
4. Streptavidin:HRP Conjugate - 120 ul
5. Sample Diluent - 20 ml
6. Biotin Antibody Dilution Buffer - 10 ml
7. HRP Conjugate Dilution Buffer - 10 ml
8. (20X) Wash Buffer - 25 ml
9. TMB Substrate - 12 ml
10. Stop Solution - 12 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. Clean tubes and Eppendorf tubes
6. Precision single and multi-channel pipette and disposable tips.
7. 37°C incubator
8. Timer.

**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 (18-25°C). 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.

**Sample Preparation and Storage:**

Specimens should be clear and non-hemolyzed.

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 (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.

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

**Test Sample Preparation** - Samples have to be diluted 1:1 (v/v), e.g. for 1:1 (100 ul sample + 100 ul Sample Diluent, provided in the kit) prior to assay to ensure reduced matrix effect. The samples may be kept at 2 - 8°C for up to three days. Long-term storage maybe stored at -20°C.

**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. **Biotinylated NOX5 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 NOX5 Antibody (concentrated) with Biotin Antibody Dilution Buffer at 1:100 and mix them thoroughly (i.e. Add 1 ul Biotinylated NOX5 Antibody into 99 ul Biotin Antibody Dilution Buffer).
5. **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).
6. **Standards Preparation:** Reconstitute original NOX5 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
1600 pg/ml	Standard No.8	Reconstitute with 1 ml Sample Diluent
800 pg/ml	Standard No.7	300 ul Standard No.8 + 300 ul Sample Diluent
400 pg/ml	Standard No.6	300 ul Standard No.7 + 300 ul Sample Diluent
200 pg/ml	Standard No.5	300 ul Standard No.6 + 300 ul Sample Diluent
100 pg/ml	Standard No.4	300 ul Standard No.5 + 300 ul Sample Diluent
50 pg/ml	Standard No.3	300 ul Standard No.4 + 300 ul Sample Diluent
25 pg/ml	Standard No.2	300 ul Standard No.3 + 300 ul Sample Diluent
0 pg/ml	Standard No.1	300 ul 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 NADPH oxidase 5, NOX5. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human NADPH oxidase 5, NOX5 present in the sample.
3. Human NADPH oxidase 5, NOX5 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 ( $\text{NaN}_3$ ), as it could destroy the HRP activity resulting in under-estimation of the amount of Human NADPH oxidase 5, NOX5.
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** and **diluted Samples** to respective wells.
3. Cover the plate with a sealer and incubate for 90 minutes at 37°C.
4. 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.
5. Pipette **100 ul Biotinylated NOX5 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. Mix well.
9. Cover the plate with a sealer and incubate for 30 minutes at 37°C.
10. Aspirate and wash as per Step (4) above.
11. Pipette **100 ul TMB Substrate** in all the wells.
12. 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.

13. Pipette **100 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 NADPH oxidase 5, NOX5 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 NADPH oxidase 5, NOX5 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 (2<sup>nd</sup> order) is best recommended for automated results.

#### Note:

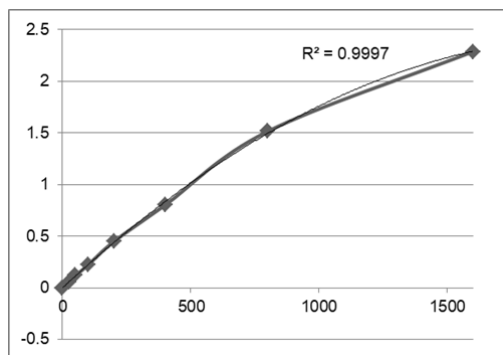
It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.

Typical Data

Standards (pg/ml)	Mean Absorbance	Corrected Absorbance
0	0.126	0
25	0.183	0.057
50	0.244	0.118
100	0.348	0.222
200	0.574	0.448
400	0.928	0.802
800	1.642	1.516
1600	2.414	2.288

Typical Graph



#### 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:

25 pg/ml - 1600 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 15 pg/ml.

**Specificity:**

The kit uses monoclonal antibodies specific for NOX5. The standards used in the kit are recombinant proteins for NOX5.

**Recovery**

Matrices listed below were spiked with certain level of NOX5 and the recovery rates were calculated by comparing the measured value to the expected amount of NOX5 in samples.

**Recovery**

Matrices listed below were spiked with certain level of TAT and the recovery rates were calculated by comparing the measured value to the expected amount of TAT in samples.

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	87-102	93
EDTA Plasma (n=5)	89-100	97
Heparin Plasma (n=5)	88-101	95

**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 TAT 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)	85-105%	87-103%	87-104%
EDTA Plasma (n=5)	85-99%	85-99%	82-100%
Heparin Plasma (n=5)	83-100%	81-95%	81-100%

**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.

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## Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A	Standard No.1			
2A	Standard No.1			
1B	Standard No.2			
2B	Standard No.2			
1C	Standard No.3			
2C	Standard No.3			
1D	Standard No.4			
2D	Standard No.4			
1E	Standard No.5			
2E	Standard No.5			
1F	Standard No.6			
2F	Standard No.6			
1G	Standard No.7			
2G	Standard No.7			
1H	Standard No.8			
2H	Standard No.8			
3A	Sample			
4A				
3B	Sample			
4B				

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## KEY SYMBOLS

	Coated Microtiter Plate (8x12 wells)
	Standard
	Biotinylated Antibody
	Conjugate Horseradish Peroxidase
	Biotin Antibody Dilution Buffer
	HRP Conjugate Dilution Buffer
	Sample Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature