






Human Neutrophil Extracellular traps, NETs GENLISA™ ELISA

REF : KBH4548

Ver 1.0


RUO

Enzyme Immunoassay for the Quantitative Determination of Neutrophil Extracellular traps, NETs 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

For Research Use Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.

REF KBH4548

 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 Neutrophil Extracellular traps, NETs GENLISA™ ELISA kit is used as an analytical tool for quantitative determination of Human Neutrophil Extracellular traps, NETs 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 Neutrophil Extracellular traps, NETs 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 Neutrophil Extracellular traps, NETs in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Human Neutrophil Extracellular traps, NETs Antibody Coated Microtiter Plate (8 x 12 wells) - 1 no
2. Human Neutrophil Extracellular traps, NETs Standard (lyophilized, concentrated, 10 ng/ml) - 2 vials
3. Biotinylated NETs Antibody (concentrated) - 120 ul
4. Streptavidin:HRP Conjugate (concentrated) - 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. 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. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
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.
6. **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 sample diluent, and several trials may be necessary. The test sample must be well mixed with the sample diluent. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with the Sample Diluent.

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 NETs 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 NETs Antibody (concentrated) with Biotin Antibody Dilution Buffer at 1:100 and mix them thoroughly (i.e. Add 1 ul Biotinylated NETs 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 Human Neutrophil Extracellular traps, NETs 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
10 ng/ml	Standard No.8	Reconstitute with 1.0 ml Sample Diluent
5 ng/ml	Standard No.7	300 ul Standard No.8 + 300 ul Sample Diluent
2.5 ng/ml	Standard No.6	300 ul Standard No.7 + 300 ul Sample Diluent
1.25 ng/ml	Standard No.5	300 ul Standard No.6 + 300 ul Sample Diluent
0.625 ng/ml	Standard No.4	300 ul Standard No.5 + 300 ul Sample Diluent
0.312 ng/ml	Standard No.3	300 ul Standard No.4 + 300 ul Sample Diluent
0.156 ng/ml	Standard No.2	300 ul Standard No.3 + 300 ul Sample Diluent
0 ng/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 Neutrophil Extracellular traps, NETs. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Neutrophil Extracellular traps, NETs present in the sample.
3. Human Neutrophil Extracellular traps, NETs 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 Neutrophil Extracellular traps, NETs.
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.
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 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 NETs 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 Neutrophil Extracellular traps, NETs 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 Neutrophil Extracellular traps, NETs 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:

0.156 ng/ml – 10 ng/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 0.094 ng/ml.

Specificity:

This assay has high sensitivity and excellent specificity for detection of NETs. No significant cross-reactivity or interference between NETs and analogues was observed.

Recovery

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

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	91-104	97
EDTA Plasma(n=5)	90-100	95
Heparin Plasma(n=5)	94-105	99

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 Neutrophil Extracellular traps, NETs 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)	87-104%	91-100%	87-99%
EDTA Plasma(n=5)	88-98%	90-101%	90-98%
Heparin Plasma(n=5)	88-95%	86-99%	93-100%

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.



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				

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 Products; 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 Products 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 Products 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 Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.















Krishgen Biosystems. 2023

THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS®, GENLISA®, DHARMAPLEX™, GENBULK™, GENLISA™, KRISHZYME®, KRISHGEN®, KRIBIOLISA®, KRISHPLEX®, TITANIUM®, QUALICHEK® are registered trademarks of KRISHGEN BIOSYSTEMS. ©KRISHGEN BIOSYSTEMS. ALL RIGHTS RESERVED.

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

	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