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GENLISA Rat Nicotinamide/Nicotinic Acid Mononucleotide Adenylyltransferase 1 (NMNAT1) ELISA

REF : KLR2487

Ver 1.0

PackSize: 96 wells

For Research Use Only

Enzyme Immunoassay for the estimation of Rat Nicotinamide/Nicotinic Acid Mononucleotide Adenylyltransferase 1 (NMNAT1) in serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids as validated

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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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 competitive ELISA technique.

Intended Use:

Enzyme Immunoassay for the estimation of Rat Nicotinamide/Nicotinic Acid Mononucleotide Adenylyltransferase 1 (NMNAT1) in serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids as validated

Principle:

This ELISA is a sandwich immunoassay. Antibodies are coated on 96 well plates. The antigen protein present in sample and standard respectively bind to the coated wells. The wells are washed and an antibody:HRP Conjugate is added which binds to the bound complex in the well. Washing is performed to remove any unbound material. TMB substrate is added and the enzyme reaction is stopped by dispensing of stop solution into the wells. The optical density (OD) of the solution at 450 nm is directly proportional to the amount of antigen protein present in the standard or samples.

Materials Provided:

1. Coated Microtiter Plate (96 wells) - 1 no
2. Standard (lyophilized, concentrated, *conc indicated on vial label) - 2 vials
3. Biotinylated Protein (concentrated) – 120 ul
4. Streptavidin:HRP Conjugate (concentrated) - 120 ul
5. Standard Diluent - 20 ml
6. Biotin Dilution Buffer - 12 ml
7. HRP Conjugate Dilution Buffer - 12 ml
8. (25X) Wash Buffer - 30 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 (18-25ºC). 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. **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.
5. **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

Please note the kit is validated for use with diluted serum and plasma samples. It is recommended to dilute 1:2 (Sample: Sample Diluent) prior to using in the assays. The user should estimate the concentration of the target protein in the test sample and choose an appropriate dilution factor to ensure that the final concentration falls within the kit's optimal detection range. Multiple trial dilutions may be required to achieve accurate results.

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) 750 ml;** dilute **30 ml of (25X) Wash Buffer in 720 ml of DI water**.
4. **Streptavidin:HRP Conjugate & Biotinylated Protein Working Solution** - Briefly spin or centrifuge the streptavidin:HRP Conjugate & Biotinylated Protein before use. Dilute them to the working concentration 100-fold with HRP Conjugate Dilution Buffer & Biotin Antigen Dilution Buffer, respectively.
5. **Standards Preparation**: Reconstitute original Standard with 1.0 ml of Standard 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. Note the Concentration is indicated on the vial label provided.
   ASSAY RANGE FOR STANDARDS PREPARATION: **31.25 - 2000 pg/ml**
   STANDARDS TO BE PREPARED: **0; 31.25; 62.5; 125; 250; 250; 500; 1000; 2000 pg/ml**

Standard Vial	Dilution Particulars
Original Standard	Reconstitute with 1.0 ml Standard Diluent
Standard No.6	Calculate and prepare as concentration indicated on the vial label
Standard No.5	300 ul Standard No.6 + 600 ul Standard Diluent
Standard No.4	300 ul Standard No.5 + 600 ul Standard Diluent
Standard No.3	300 ul Standard No.4 + 600 ul Standard Diluent
Standard No.2	300 ul Standard No.3 + 600 ul Standard Diluent
Standard No.1	600 ul Standard 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 the analyte, High Dose
3. The analyte 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 analyte in the sample.
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 diluted Samples** to respective wells.

3. Pipette **100 ul Biotinylated Protein Working Solution** to all wells.

4. Cover the plate with a sealer and incubate for 60 minutes at 37°C.

5. 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.

6. Pipette **100 ul Streptavidin:HRP Conjugate Working Solution** to all wells. Mix well.

7. Cover the plate with a sealer and incubate for 30 minutes at 37°C.

8. Aspirate and wash as per Step (5) above.

9. Pipette **100 ul TMB Substrate** in all the wells.

10. Incubate the plate at **37°C** for **10 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision.

11. Pipette **100 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

12. 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 450 nm) 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 Analyte 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 Analyte 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:
31.25 - 2000 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 ***18.75 pg/ml***

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

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

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	81-101	95
EDTA Plasma(n=5)	85-96	92
Heparin Plasma(n=5)	78-90	85

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 the analyte 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)	86-99%	91-104%	85-95%	81-92%
EDTA Plasma(n=5)	80-90%	78-89%	91-103%	85-96%
Heparin Plasma(n=5)	92-103%	83-97%	81-92%	89-99%

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.
- 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	Sample Sample			
1H 2H	Sample Sample			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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