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GENLISA® Rat Kelch Like Ech Associated Protein 1 (KEAP1) ELISA

REF : KLR2124

Ver 1.0

RUO

Enzyme Immunoassay for the estimation of Rat Kelch Like Ech Associated Protein 1 (KEAP1) ELISA 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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REF **KLR2124** **96 tests**

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Introduction:

Enzyme Immunoassay for the estimation of Rat Kelch Like Ech Associated Protein 1 (KEAP1) ELISA in serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids as validated

This GENLISA Rat Kelch Like Ech Associated Protein 1 (KEAP1) ELISA includes features like:
- Ready to use protocol with break-apart wells for ease of use
- Standardisation and High Reproducibility
- Lot to Lot Consistency
- Accuracy and Precision

Validated against seven points for a “GOLD RING” Standard Quality ELISA - the benchmark sign for Krishgen Quality. This This GENLISA ELISA kit is used for assessing the specific biomarker in samples analytes which may be serum, plasma, tissue homogenates and other biological fluids. The kit uses monoclonal antibodies to ensure a high degree of sensitivity and specificity in the estimation of the analyte.

ELISA Type:
Sandwich ELISA, Double Antibody

Pack Type:
1 x 96 wells, break-apart wells

Species Reactivity:
Rat

Alternate Names / Synonyms:
INrf2; KLHL19; Cytosolic inhibitor of Nrf2; Kelch-like protein 19;

Intended Use:

The GENLISA® Rat Kelch Like Ech Associated Protein 1 (KEAP1) ELISA is used as an analytical tool for quantitative determination of GENLISA® Rat Kelch Like Ech Associated Protein 1 (KEAP1) ELISA in serum, plasma and other biological samples.

Principle Of Assay:

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.

Detection Method:

Colorimetric, Absorbance measured at 450nm

Conjugate-Enzyme Reaction:

Uses stable HRP Enzyme Conjugate with (single component) TMB Chromogenic Substrate for color development.

Regulatory Status:

Research Use Only.

Sample Type:

Serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and the anaylyte 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 analyte in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

- Coated Microtiter Plate (96 wells) - 1 no
- Standard (lyophilized, concentrated) - 2 vials
- Biotinylated Antibody (concentrated) - 120 ul
- Streptavidin:HRP Conjugate (concentrated) - 120 ul
- Standard Diluent - 20 ml
- Biotin Antibody Dilution Buffer - 12 ml
- HRP Conjugate Dilution Buffer - 12 ml
- (20X) Wash Buffer - 25 ml
- TMB Substrate - 12 ml
- Stop Solution - 12 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.
- **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

Please note the kit is validated for use with neat samples.
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. Dilute the sample using PBS. Multiple trial dilutions may be required to achieve accurate results.

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) 500 ml;** dilute **25 ml of (20X) Wash Buffer in 475 ml of DI water**.
- **Streptavidin:HRP Conjugate & Biotinylated Antibody Working Solution** - Briefly spin or centrifuge the Streptavidin:HRP Conjugate & Biotinylated Antibody before use. Dilute them to the working concentration 100-fold with HRP Conjugate Dilution Buffer & Biotin Antibody Dilution Buffer, respectively.
- **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.

Standard Concentration	Standard Vial	Dilution Particulars
1x	Standard No.8	Reconstitute with 1.0 ml Standard Diluent
1:1	Standard No.7	500 ul Standard No.8 + 500 ul Standard Diluent
1:1	Standard No.6	500 ul Standard No.7 + 500 ul Standard Diluent
1:1	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent
1:1	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent
1:1	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent
1:1	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent
1:1	Standard No.1	500 ul Standard Diluent only

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 the analyte. High Dose Hook Effect is due to excess of antibody for very high concentrations of the analyte present in the sample.
- The analyte 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 analyte present in the sample.

- 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 Samples to respective wells.

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

- Aspirate the plate. DO NOT WASH.

- Pipette 100 ul Biotinylated Antibody Working Solution to all wells.

- Cover the plate with a sealer and incubate for 60 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 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 (7) above.

- Pipette 100 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 100 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 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 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.

ELISA Interpretation:

Quantitative Results Intepretation, 4PL (2nd order) or Cubic Spline Graph. Software (MS Excel) available, on request, to support interpretation of results.

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.

Assay Range:
15.625 - 1000 pg/ml

Standards Available:
0; 15.625; 31.25; 62.5; 125; 125; 250; 500; 1000 pg/ml

Calibration Curve:
8 point; standards available

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 9.375 pg/ml.

Specificity:
The assay uses monoclonal antibody against the analyte and is highly specific as a capture protein.

Interference:
Preparations of the known factors were assayed for interference. No significant interference was observed.

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

Matrix	Recovery Range (%)	Average (%)
serum(n=5)	93-105	99
EDTA plasma(n=5)	90-99	95
heparin plasma(n=5)	85-97	91

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 GENLISA® Rat Kelch Like Ech Associated Protein 1 (KEAP1) ELISA 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)	91-99%	85-92%	86-104%	80-94%
EDTA plasma(n=5)	94-107%	87-105%	80-93%	84-98%
heparin plasma(n=5)	83-96%	81-96%	94-106%	79-104%

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	Standard No.7 Standard No.7			
1H 2H	Standard No.8 Standard No.8			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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SYMBOLS KEY

MTP	Coated Microtiter Plate (96 wells)
STD	Standard
BIOTIN AB	Biotinylated Antibody
HRP CONJ	Conjugate Horseradish Peroxidase
BIOTIN DIL	Biotin Antibody Dilution Buffer
HRP DIL	HRP Conjugate Dilution Buffer
STD DIL	Standard Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature