Rat Insulin, INS GENLISA™ ELISA

REF : KLR0707

Ver 3.3

RUO

Enzyme Immunoassay for the Quantitative Determination of Insulin in Rat 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 KLR0707 Σ 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

Rat Insulin GENLISA™ ELISA

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.

Long Name: Insulin-like Growth Factor I/Insulin-like Growth Factor 1

Entrez Gene IDs: 3479 (Human); 16000 (Mouse); 24482 (Rat)

Alternate Names: IBP1; IGF1; IGF-1; IGF1A; IGFI; IGF-I; IGF-IA; IGF-IB; insulin-like growth factor 1 (somatomedin C); insulin-like growth factor 1; insulin-like growth factor I; insulin-like growth factor IA; insulin-like growth factor IB; Mechano growth factor; MGF; Somatomedin A; Somatomedin C; somatomedin-C

Intended Use:

The Rat Insulin GENLISA™ ELISA kit is used as an analytical tool for quantitative determination of Rat Insulin in serum, plasma and other biological samples.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing rat Insulin react with already coated affinity purified capture anti-Rat Insulin antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-Rat Insulin is added leading to formation of a sandwich complex of solid phase antibody-Rat Insulin-biotin labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure. Streptavidin:HRP conjugate is added which binds to Biotinylated Anti-Rat Insulin complex. The wells are washed to remove any unbound reactants as per the wash procedure. The substrate 3, 3',5, 5' Tetra Methyl Benzidine is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and it is directly proportional to the concentration of Rat Insulin present in the samples.

Materials Provided:

1. Rat Insulin monoclonal antibody coated microtiter plate (12x8 wells) - 1 no
2. Recombinant Rat Insulin Standard lyophilized, 24 mIU/ml - 2 vials
3. (1X) Standard Diluent - 10 ml
4. Anti-Rat Insulin:HRP Conjugate - 12 ml
5. (20X) Wash Buffer - 25 ml
6. (1X) Assay Diluent - 12 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. 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. After reconstitution of standard, prepare aliquots and store the aliquots at -20 ºC.
3. All the reagents and wash solutions should be used within 12 months from manufacturing date.

4. 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.
5. 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, re-centrifuge.
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, re-centrifuge.

4. Serum and Plasma samples should be diluted **1:100 (v/v)** for optimal recovery, (for example 1 ul sample + 99 ul Assay Diluent (1X) prior to assay. In cases where matrix interferences is under or over observed, the samples may be diluted with Assay Diluent (1X) accordingly.

The samples may be kept at 2 - 8°C for up to three days. For long-term storage please store at -20°C.

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

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) 500 ml;** dilute **25 ml of (20X) Wash Buffer in 475 ml of DI water**.
4. **Standards Preparation**: Reconstitute the recombinant protein by adding 100 µl of Standard Diluent (1X) to give a concentration of 24 mIU/ml. Keep the standard for 15 mins with gentle agitation before making further dilutions. Add 40 ul of reconstituted standard (24mIU/ml) to 460 ul Standard Diluent (1X) to prepare 1920 uIU/ml standard solution. This is the top standard. Thus the Rat Insulin Standards concentrations are 1920uIU/ml, 960uIU/ml, 480uIU/ml, 240uIU/ml, 120uIU/ml, 60uIU/ml and 30uIU/ml. Standard Diluent (1X) serves as the zero standard (0 uIU/ml).

Standard Concentration	Standard No	Dilution Particulars
24 mIU/ml	Standard, concentrated	Original Standard (lyophilized) + 100ul Standard Diluent (1X)
1920uIU/ml	Standard No.7	40 ul Original Standard + 460 ul Standard Diluent (1X)
960uIU/ml	Standard No.6	250 ul Standard No. 7 + 250 ul Standard Diluent (1X)
480uIU/ml	Standard No.5	250 ul Standard No. 6 + 250 ul Standard Diluent (1X)
240uIU/ml	Standard No.4	250 ul Standard No. 5 + 250 ul Standard Diluent (1X)
120uIU/ml	Standard No.3	250 ul Standard No. 4 + 250 ul Standard Diluent (1X)
60uIU/ml	Standard No.2	250 ul Standard No. 3 + 250 ul Standard Diluent (1X)
30uIU/ml	Standard No.1	250 ul Standard No. 2 + 250 ul Standard Diluent (1X)
0 uIU/ml	Standard No.0	250 ul Standard Diluent (1X)

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 Rat Insulin. High Dose Hook Effect is due to excess of antibody for very high concentrations of Rat Insulin present in the sample.
3. Rat Insulin 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 Rat Insulin.
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** of **Standards** and **Samples** to the plate, Seal plate and incubate for 1 hour at RT.

3. Aspirate and wash plate 4 times with **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. All the washes should be performed similarly.

4. Add **100 ul** of **Anti-Rat Insulin:HRP Conjugate** solution to each well, Seal plate and incubate for 1 hour at RT.

5. Wash plate 4 times with **Wash Buffer (1X)** as in step 3.

6. Add 100 ul of **TMB Substrate** solution and incubate in the dark for 30 minutes at RT. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

7. Stop reaction by adding 100 ul of **Stop Solution** to each well. Positive wells should turn from blue to yellow.

8. Read absorbance at 450 nm within 30 minutes of stopping reaction.

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 Rat Insulin 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 Rat Insulin Concentration.

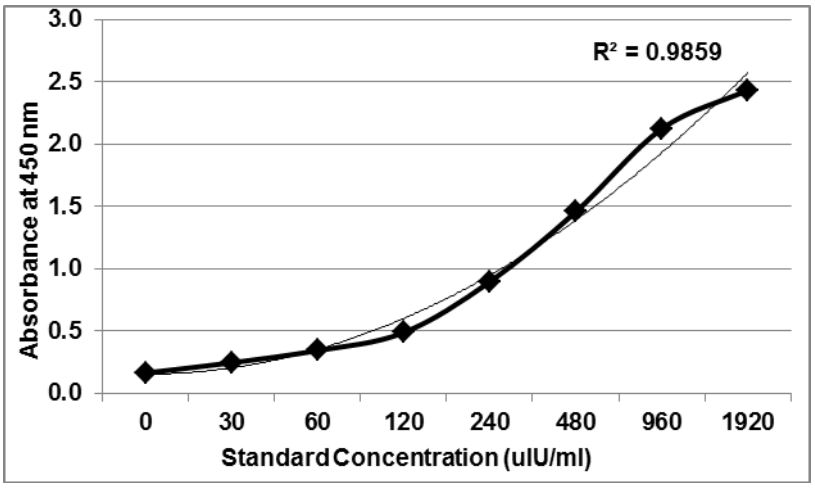
If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

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.

Typical Data

Standard Concentration (uIU/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.166	--	--
30	0.248	34.0	113.4
60	0.346	71.2	118.7
120	0.490	117.2	97.6
240	0.897	242.2	100.9
480	1.459	463.3	96.5
960	2.126	1020.7	106.3
1920	2.436	1823.7	95.0

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:
30 uIU/ml - 1920 uIU/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 30 uIU/ml.

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

Cross Reactivity:
This assay recognizes natural and recombinant rat IL-6. The factors listed below were prepared at 500 pg/ml in (1X) Assay Diluent and assayed for cross-reactivity. No significant cross-reactivity or interference was observed

Recombinant mouse:
IGFBP-2 IGFBP-5 IGFBP-6 IGF-II

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

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	90-101	95
EDTA Plasma(n=5)	85-97	90
Heparin Plasma(n=5)	81-92	86

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 Insulin 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)	87-98%	80-95%	88-103%	89-101%
EDTA Plasma(n=5)	94-105%	93-106%	78-99%	97-107%
Heparin Plasma(n=5)	91-101%	86-99%	82-93%	79-89%

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 Rat 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.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

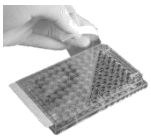
Components NOW (2-8ºC) —30 mins / RT Before Use→  Thaw at Room Temperature (18-24ºC) → USE

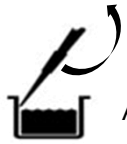
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

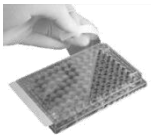
3.  Pipette **100 ul Standards** into respective Standard wells.

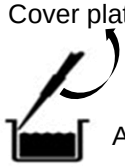
4.  Pipette **100 ul Samples** into the sample wells.

5. Cover plate  and incubate for  at room temperature.

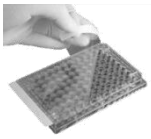
6.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

7.  Pipette **100 ul** diluted **Anti-Rat Insulin:HRP Conjugate** to all wells.

8. Cover plate  and incubate for  at room temperature.

9.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

10.  Pipette **100 ul TMB Substrate** into each wells

11. Cover plate  and incubate for  at room temperature.

12.  Pipette **100 ul Stop Solution** into each well.

13. Read absorbance at 450nm with a  microplate reader within  of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Standard No.0 Standard No.0			
1B 2B	Standard No.1 Standard No.1			
1C 2C	Standard No.2 Standard No.2			
1D 2D	Standard No.3 Standard No.3			
1E 2E	Standard No.4 Standard No.4			
1F 2F	Standard No.5 Standard No.5			
1G 2G	Standard No.6 Standard No.6			
1H 2H	Standard No.7 Standard No.7			
3A 4A	Sample			
3B 4B	Sample			

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. 2024

THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	Coated Microtiter Plate (12x8 wells)
STD	Rat Insulin Standard, lyophilized
HRP CONJ	HRP Conjugate
1X STD DIL	(1X) Standard Diluent
1X ASY DIL	(1X) Assay 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