

GENLISA™ Human Interleukin 15 (IL-15 / IL15) ELISA

REF : KBH0097

Ver 3.3

RUO

Enzyme Immunoassay for the Quantitative Determination of Human IL-15 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

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96 tests

**KRISHGEN BioSystems**

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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 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.

Long Name: Interleukin 15

Entrez Gene IDs: 3600 (Human); 16168 (Mouse); 25670 (Rat); 102119613 (Cynomolgus Monkey); 493682 (Feline)

Alternate Names: IL15; IL-15; IL-15MGC9721; interleukin 15; interleukin-15

Intended Use:

The GENLISA™ Human Interleukin 15 (IL-15 / IL15) ELISA kit is used as an analytical tool for quantitative determination of Human IL-15 in serum, plasma and other biological samples.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing Human IL-15 react with already coated affinity purified capture anti-Human IL-15 antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-Human IL-15 is added leading to formation of a sandwich complex of solid phase antibody-Human IL-15 -biotin labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure. Streptavidin:HRP is added which binds to Biotinylated Anti-Human IL-15 complex. The wells are washed to remove any unbound reactants as per the wash procedure. The substrate 3,3',5,5' Tetra Methyl Benzidine (TMB) is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader at 450 nm and it is directly proportional to the concentration of Human IL-15 present in the samples

Materials Provided:

1. Anti-Human IL-15 Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Recombinant Human IL-15 Standard (70 ng/ml; lyophilized) - 2 vials
3. Anti-Human IL-15 Biotin Conjugated Detection Antibody (30 ug/ml; lyophilized) - 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial
5. (1X) Assay Diluent - 50 ml
6. (20X) Wash Buffer - 25 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 pipette to measure volumes ranging from 1 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Clean tubes and Eppendorf tubes.
7. Precision single and multi-channel pipette and disposable tips.
8. 37°C incubator.
9. 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. Store Recombinant Human IL-15 Standard and Anti-Human IL-15 Biotin Conjugated Detection Antibody at 2-8°C. After reconstitution aliquot recombinant proteins into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.
4. 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.
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.

**Specimen Collection and Handling:**

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at temperature <-20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum layer and assay immediately or store serum samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or store plasma samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

Reagent Preparation:

Please refer to lot specific instructions for preparation of the reagents.

Assay Procedure:

1. Bring 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. **Standards Preparation:** Reconstitute the lyophilized vial with 30 ul of Assay Diluent (1X) to generate a 70 ng/ml. Dilute 14.29 ul of reconstituted Standard (70 ng/ml) with 985.71 ul of Assay Diluent (1X) to generate a 1000 pg/ml top standard. Perform serial dilutions by using 1000 pg/ml top standard as per the below table. Thus, the Human IL-15 standards are 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml and 15.6 pg/ml. Assay Diluent (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
70 ng/ml	Reconstituted Standard	Lyophilized Standard provided in the Kit + 30 ul Assay Diluent (1X)
1000 pg/ml	Standard No.7	14.29 ul Reconstituted Standard + 985.71 ul Assay Diluent (1X)
500 pg/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay Diluent (1X)
250 pg/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay Diluent (1X)
125 pg/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay Diluent (1X)
62.5 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay Diluent (1X)
31.25 pg/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay Diluent (1X)
15.6 pg/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay Diluent (1X)
0 pg/ml	Standard No.0	Only Assay 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 Human IL-15. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human IL-15 present in the sample.
3. Human IL-15 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 (NaN₃), as it could destroy the HRP activity resulting in under-estimation of the amount of Human IL-15.
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.
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 each well, Seal plate and incubate for 2 hours at Room Temperature (18-25°C).
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 diluted **Biotinylated Detection Antibody** solution to each well, seal plate and incubate for 2 hour at Room Temperature (18-25°C).
5. Wash plate 4 times with **Wash Buffer (1X)** as in step 3.
6. Add **100 ul** of diluted **Streptavidin:HRP** solution to each well, seal plate and incubate for 30 minutes at Room Temperature (18-25°C).
7. Wash plate 4 times with **Wash Buffer (1X)** as in step 3.
8. Add **100 ul** of **TMB Substrate** solution and incubate in the dark for 30 minutes at Room Temperature (18-25°C). Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.
9. Stop reaction by adding **100 ul** of **Stop Solution** to each well. Positive wells should turn from blue to yellow.
10. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

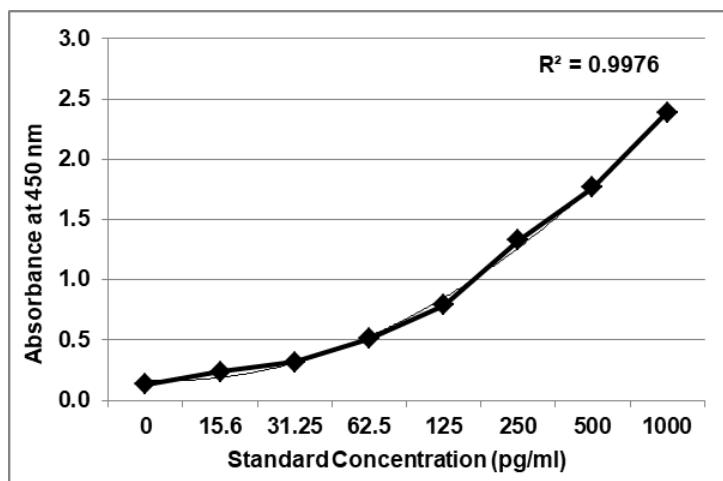
Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Human IL-15 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 unknown Human IL-15 concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4PL (2nd order) is best recommended for automated results.

Typical Data (representative only)

Standard Concentration (pg/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.128	0.141	0.134	1.0	--
15.6	0.222	0.255	0.238	16.3	104.2
31.25	0.325	0.312	0.319	29.0	92.7
62.5	0.496	0.528	0.512	62.4	99.8
125	0.783	0.798	0.790	119.3	95.5
250	1.355	1.300	1.327	272.1	108.8
500	1.869	1.666	1.768	469.9	94.0
1000	2.337	2.444	2.390	1021.2	102.1

Typical Graph (representative only)

**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:

15.6 pg/ml - 1000 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:

This assay has high sensitivity and excellent specificity for detection of IL-15. No significant cross-reactivity or interference between IL-15 and analogues was observed.

Recovery

Matrices listed below were spiked with certain level of IL-15 and the recovery rates were calculated by comparing the measured value to the expected amount of IL-15 in samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	89-102	97
EDTA Plasma(n=5)	90-99	94
Heparin Plasma(n=5)	90-104	98

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 IL-15 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)	87-103%	88-99%	86-98%
EDTA Plasma(n=5)	85-99%	82-100%	83-96%
Heparin Plasma(n=5)	80-98%	82-89%	84-95%

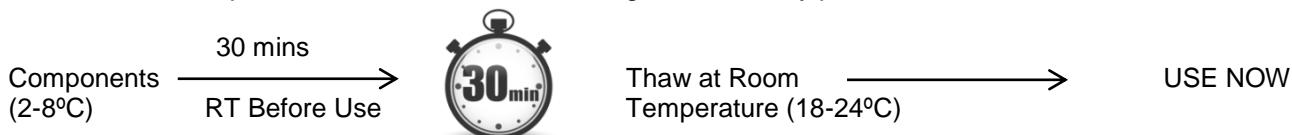
Safety Precautions:

- **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/v) 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.

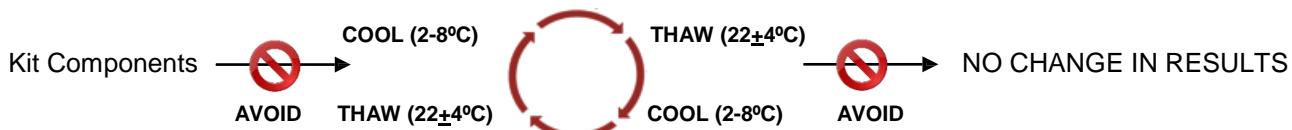


SCHEMATIC ASSAY PROCEDURE

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



3. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the 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 **Biotinylated Detection Antibody** 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** of diluted **Streptavidin:HRP** to all wells.

11. Cover plate and incubate for at room temperature

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

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

14. Cover plate and incubate for at room temperature.

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

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

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SYMBOLS KEY

	Anti-Human IL-15 Antibody Coated Microtiter Plate (12 x 8 wells)
	Recombinant Human IL-15 Standard, Lyophilized
	Anti-Human IL-15 Biotin Conjugated Detection Antibody, Lyophilized
	Concentrated Streptavidin Horseradish Peroxidase
	(1X) Assay Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalogue Number
	Expiration Date
	Storage Temperature