GENLISA™ Human Interleukin 17 (IL-17 / IL17) ELISA

REF : KBH0142

Ver 4.4

RUO

NIBSC Calibrated Assay

*the standards used in this kit are calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK.
1 ng of supplied standard equals 12 U of 01/420 NIBSC-standard. Please note that the calibration is lot specific.

Enzyme Immunoassay for the Quantitative Determination of IL-17 in humanserum, 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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REF KBH0142 **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: sales1@krishgen.com | http://www.krishgen.com

GENLISA™ Human Interleukin 17 (IL-17 / IL17) 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 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 17

Entrez Gene IDs: 3605 (Human); 16171 (Mouse); 301289 (Rat); 449530 (Porcine); 481837 (Canine); 102119976 (Cynomolgus Monkey)

Alternate Names: CTLA8; CTLA-8; CTLA8cytotoxic T-lymphocyte-associated serine esterase 8; Cytotoxic T-lymphocyte-associated antigen 8; IL17; IL-17; IL17A; IL-17A; IL-17Acytotoxic T-lymphocyte-associated protein 8; IL-17CTLA-8; IL17interleukin-17A; interleukin 17 (cytotoxic T-lymphocyte-associated serine esterase 8); interleukin 17A

Intended Use:

The GENLISA™ Human Interleukin 17 (IL-17 / IL17) ELISA kit is used as an analytical tool for quantitative determination of Human IL- 17 in serum, plasma and other biological samples.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing human IL-17 react with already coated affinity purified capture Anti-Human IL-17 antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-human IL-17 is added leading to formation of a sandwich complex of solid phase antibody-human IL-17-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-human IL-17 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-17 present in the samples.

Materials Provided:

1. Anti-Human IL-17 Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Recombinant Human IL-17 Standard (lyophilized, 1 ug/ml) - 2 vials
3. Anti-Human IL-17 Biotin Conjugated Detection Antibody - 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase -1 vial
5. 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 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 minutes 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 minutes at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Urine-** Collect urine in a sterile container, centrifuge for 20 minutes at 2000-3000 rpm. Remove the supernatant.If precipitation appears, recentrifuge.
5. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20 minutes 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 minutes 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 (pH 7.4) 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 dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples contain very high concentrations of the analyte, dilute the samples with PBS first and then do further dilutions of the samples with the provided Assay Diluent in the kit.

Reagent Preparation:

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

Assay Procedure:

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. **Standards Preparation: :** Reconstitute the lyophilized vial with 20 ul of Distilled water to generate a 1 ug/ml. Dilute 5 ul of reconstituted Standard (1 ug/ml) with 495 ul of Assay diluent to generate a 10 ng/ml middle stock solution. Perform serial dilutions by using middle stock solution as per the below table. Thus, the Human IL-17 standards are 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, and 31.25 pg/ml. Assay Diluent serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
1 ug/ml	Reconstituted Standard	Lyophilized Standard provided in the Kit + 20 ul of Distilled Water
10 ng/ml	Middle stock	5 ul Reconstituted Standard + 495 ul Assay diluent
2000 pg/ml	Standard No.7	200 ul Middle Stock + 800 ul Assay diluent
1000 pg/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay diluent
500 pg/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay diluent
250 pg/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay diluent
125 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay diluent
62.5 pg/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay diluent
31.25 pg/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay diluent
0 pg/ml	Standard No.0	Assay 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 IL-17. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human IL-17 present in the sample.
3. Human IL-17 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 IL-17.
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 each well, then add 50 ul of diluted **Biotinylated Detection Antibody** to all wells mix well Seal plate and incubate for 120 minutes at 37°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 Streptavidin:HRP** solution to each well, seal plate and incubate for 30 minutes at 37°C.

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 37°C. 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 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-17 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 IL-17 Concentration.

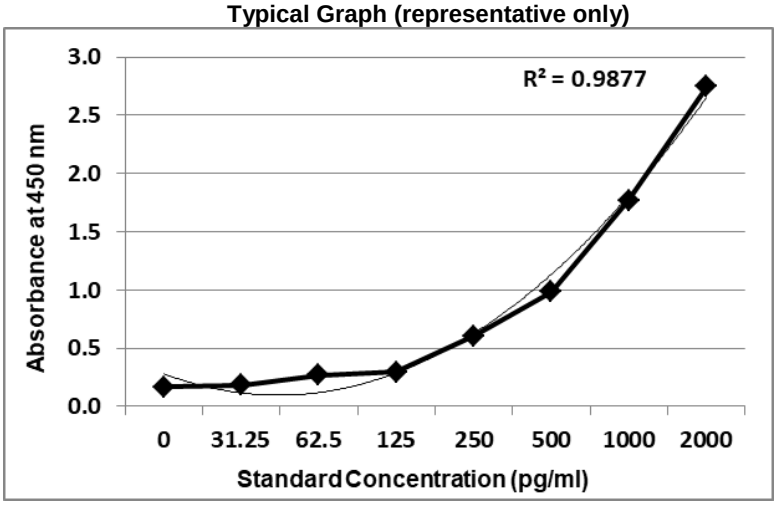
If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

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 (representative only)

Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.166	4.7	--
31.25	0.184	21.7	69.6
62.5	0.269	81.3	130.1
125	0.295	97.5	78.0
250	0.602	272.5	109.0
500	0.985	488.1	97.6
1000	1.769	1004.6	100.5
2000	2.750	1998.7	99.9

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:
31.25 pg/ml - 2000 pg/ml.

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 15.6 pg/ml.

Specificity:
This assay has high sensitivity and excellent specificity for detection of IL-17. No significant cross-reactivity or interference between IL-17 and analogues was observed.1 ng of supplied standard equals 12 U of 01/420 NIBSC-standard. Please note that the calibration is lot specific.

Cross Reactivity:
This assay recognizes natural and recombinant human IL-17a. The markers listed below were prepared at 50 ng/ml in Assay Diluent and assayed for cross-reactivity. No significant cross-reactivity or interference was observed.

Recombinant human:
IFN-γ IL-10 IL-12 IL-16 IL-17B IL-17C IL-17D IL-17F

Recovery
Matrices listed below were spiked with certain level of IL-17 and the recovery rates were calculated by comparingthe measured value to the expected amount of IL-17 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-17 and theirserial 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 notbe 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.

Components (2-8ºC) → 30 mins RT Before Use → 

→ Thaw at Room Temperature (18-24ºC) → USE NOW

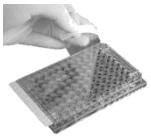
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → THAW (22±4ºC) → COOL (2-8ºC) → 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.

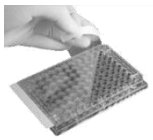
5.  Pipette **50 ul** diluted **Biotinylated Detection Antibody** to all wells mix well.

6. Cover plate  and incubate for 

at 37°C.

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

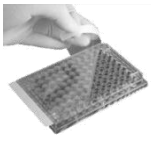
8.  Pipette **100 ul** of diluted **Streptavidin: HRP** to all wells

9. Cover plate  and incubate for 

at 37°C.

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

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

12. Cover plate  and incubate for 

at 37°C.

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

14. Read absorbance at 450 nm with a  microplate reader within 

of stopping reaction.

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.

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THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	**Anti-Human IL-17 Antibody Coated Microtiter Plate (12 x 8 wells)**
STD	**Recombinant Human IL-17 Standard, Lyophilized**
BIO AB	**Anti-Human IL-17 Biotin Conjugated Detection Antibody**
STRP HRP	**Concentrated Streptavidin Horseradish Peroxidase**
ASY DIL	**Assay Diluent**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalogue Number**
	**Expiration Date**
	**Storage Temperature**