# Human IL-17A GENLISA™ ELISA

REF: KB1079

Ver 5.2

**RUO**

ELISA for Accurate Quantitation of Human IL-17A from Cell Culture Supernatant, Serum, Plasma, or Other Bodily Fluids

| | | | |
|---|---|---|---|
| **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:**

Interleukin-17a or IL-17a is a protein that in humans is encoded by the *IL17A* gene. The protein encoded by this gene is a proinflammatory cytokine produced by activated T cells. This cytokine regulates the activities of NF-kappaB and mitogen-activated protein kinases. This cytokine can stimulate the expression of IL-6 and cyclooxygenase-2 (PTGS2/COX-2), as well as enhance the production of nitric oxide (NO). High levels of this cytokine are associated with several chronic inflammatory diseases including rheumatoid arthritis, psoriasis and multiple sclerosis.

**Intended Use:**

The human IL-17a ELISA is an enzyme-linked immunosorbent assay for accurate and precise quantitative detection of human IL-17a from samples including serum, plasma, and supernatants from cell cultures. The human IL-17a ELISA is for research use only. Not for diagnostic or therapeutic procedures.

**Materials Provided:**

1. Microtiter Coated Plate (12 X 8 wells) – 1 no
2. Recombinant Human IL-17a Standard (Lyophilized,1ug/ml) – 1 vial
3. Human IL-17a Biotin Conjugated Detection Antibody – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial
5. (20X) Wash Buffer – 25ml
6. Assay Diluent – 50ml
7. TMB Substrate – 12ml
8. Stop Solution – 12ml
9. Instruction Manual

**Materials to be provided by the End-User:**

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50µl to 1000µl.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Semi-Log graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

**Storage Information:**

1. Store main kit components at 2-8°C.
2. Store recombinant Standard at 2-8° C. Upon reconstitution, aliquot recombinant protein into polypropylene vials and store at -20°C as per assay requirements. Do not Freeze thaw for more than two times.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

**Health Hazard Warnings:**

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.

2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

**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 all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. **Standards Preparation:** Reconstitute the lyophilized vial in 20ul of Distilled water to get concentration of 1 ug/ml. Add 2ul of reconstituted standard in 998ul of Assay Diluent to prepare top standard of 2000 pg/ml. Perform serial dilutions by using main stock solution as per the below table. Thus, the Human IL-17a standard concentrations are 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml and 31.25pg/ml . Assay Diluent serves as the zero standard (0 pg/ml).

| Standard Concentration | Standard No | Dilution Particulars |
|---|---|---|
| 1 ug/ml | Standard, lyophilized | Original Standard provided in the Kit + 20ul Distilled water |
| 2000 pg/ml | Standard No.7 | 2ul Reconstituted Standard + 998 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 |

3. Add 50ul of diluted **Detection Antibody** followed by addition of 100ul/well of **Standards** and **Samples** to the plate. Seal plate and incubate at 37°C for 2 hours.

4. 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.

5. Add 100µl of diluted **Streptavidin-HRP** solution to each well, seal plate and incubate at 37ºC for 30 minutes.

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

7. Add 100µl of **TMB Substrate** solution and incubate in the dark at 37ºC for 30 minutes. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

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

9. 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. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on Semi-Log graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown cytokine concentrations, find the unknowns 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 cytokine concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

**Performance Characteristics:**
Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

**Sensitivity:**
Limit Of Quantification: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to 18.75 pg/ml.

**Specificity:**
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for human IL-17a.

**Assay Range:**
31.25 pg/ml to 2000 pg/ml

**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 Human IL-17a and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

| Sample | 1:2 | 1:4 | 1:8 |
|---|---|---|---|
| serum (n=5) | 84-107% | 87-108% | 82-112% |
| EDTA plasma (n=5) | 83-102% | 83-115% | 83-118% |
| heparin plasma (n=5) | 83-99% | 80-95% | 82-93% |

**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.

**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 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.

**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 product; 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 product 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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## SYMBOLS KEY

| | |
|---|---|
| MTP | Human IL-17a Microtiter Plate (12X8 wells) |
| STD | Human IL-17a Standard, lyophilized |
| BIO CONJ | Biotin Conjugated Detection Antibody |
| STRP HRP | 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 |