

# Human IL-5 GENLISA™ ELISA

**REF:** KB1067

Ver 1.0

**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 14 U of 90/586 NIBSC-standard. Please note that the calibration is lot specific.

ELISA for Accurate Quantitation of Human IL-5 from Cell Culture Supernatant, Serum, Plasma, or Other Bodily Fluids

<b>RUO</b>	<b>For Research Use Only</b>	<b>REF</b>	<b>Catalog Number</b>
	<b>Store At</b>	<b>LOT</b>	<b>Batch Code</b>
	<b>Manufactured By</b>		<b>Biological Risk</b>
	<b>Expiry Date</b>		<b>Consult Operating Instructions</b>

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**Introduction:**

IL-5 is a 115-amino acid (in human, 133 in the mouse) -long TH2 cytokine that is part of the hematopoietic family. Unlike other members of this cytokine family (namely interleukin 3 and GM-CSF), this glycoprotein in its active form is a homodimer.

**Long Name:** Interleukin 5

**Entrez Gene IDs:** 3567 (Human); 16191 (Mouse); 24497 (Rat); 397409 (Porcine); 280825 (Bovine); 403790 (Canine); 493803 (Feline)

**Alternate Names:** BCDF mu; B-cell differentiation factor I; BCGFII; EDF; Eo-CSF; Eosinophil differentiation factor; IL5; IL-5; IL-5T-cell replacing factor; interleukin 5 (colony-stimulating factor, eosinophil); interleukin-5; TRF; TRFB cell differentiation factor I

**Intended Use:**

The GENLISA™ Human IL-5 ELISA is specifically designed for the accurate quantitation of human IL-5 from cell culture supernatant, serum, plasma or other bodily fluids. It is ready-to-use, accurate, and sensitive.

**Principle:**

This assay is based on the Sandwich ELISA procedure. Samples containing human IL-5 react with already coated affinity purified capture anti-human IL-5 antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-human IL-5 is added leading to formation of a sandwich complex of solid phase antibody-human IL-5-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-5 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 Human IL-5 present in the samples.

**Materials Provided:**

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

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

1. Microplate Reader able to measure absorbance at 450nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. 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 reconstituting, aliquot recombinant protein into polypropylene vials and store at -20°C as per assay requirements. Do not freeze-thaw for more than two times to ensure no loss of activity.
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 duplicates. A standard curve is required for each assay.
2. **Standards Preparation:** Reconstitute the lyophilized vial with 20 ul of Distilled water to generate a 1ug/ml. Dilute 5 ul of original Standard (1ug/ml) with 495 ul of Assay diluent (1X) 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-5 standards concentration 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
1 ug/ml (Lyophilized)	Standard Main stock	Original Standard. Reconstitute in 20 ul of Distilled water
10 ng/ml	Middle stock	5 ul Original Standard + 495 ul Assay Diluent (1X)
1000 pg/ml	Standard No.7	50 ul Middle Stock + 450 ul Assay Diluent (1X)
500 pg/ml	Standard No.6	250 ul Standard No.7 + 250 ul Assay Diluent (1X)
250 pg/ml	Standard No.5	250 ul Standard No.6 + 250 ul Assay Diluent (1X)
125 pg/ml	Standard No.4	250 ul Standard No.5 + 250 ul Assay Diluent (1X)
62.5 pg/ml	Standard No.3	250 ul Standard No.4 + 250 ul Assay Diluent (1X)
31.25 pg/ml	Standard No.2	250 ul Standard No.3 + 250 ul Assay Diluent (1X)
15.6 pg/ml	Standard No.1	250 ul Standard No.2 + 250 ul Assay Diluent (1X)
0 pg/ml	Standard No.0	500 ul Assay Diluent (1X)

3. Add **100 ul** of **Standards** and **Samples** to the plate. Seal plate and incubate for 2 hours at Room Temperature (18-25°C).
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 off 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 **100 ul** of diluted **Detection Antibody** solution to each well, seal plate and incubate for 1 hour at Room Temperature (18-25°C).
6. Wash plate 4 times with **Wash Buffer (1X)** as in step 4.
7. Add **100 ul** of diluted **Streptavidin:HRP** solution to each well, seal plate and incubate for 1 hour at Room Temperature (18-25°C).
8. Wash plate 4 times with **Wash Buffer (1X)** as in step 4.
9. Add **100 ul** of **TMB Substrate** solution and incubate in the dark for 15 minutes at Room Temperature. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.
10. Stop reaction by adding **100 ul** of **Stop Solution** to each well. Positive wells should turn from blue to yellow.
11. Read absorbance at 450 nm within 30 minutes of stopping reaction.

#### Calculation of Results:

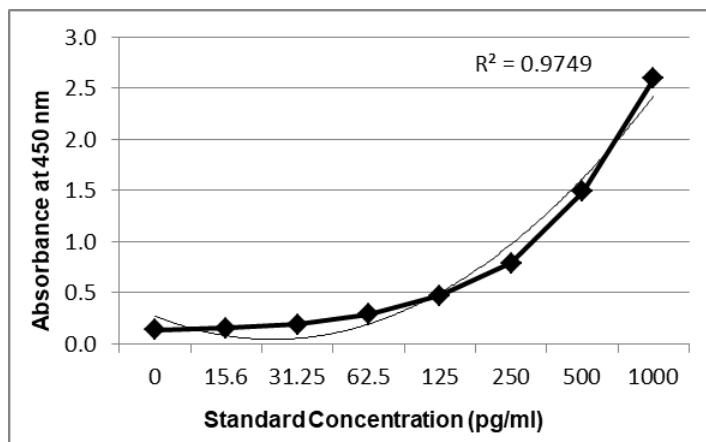
Determine the mean absorbance for each set of duplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight 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. Software which is able to generate a cubic spline curve-fit or a polynomial regression to the 2nd order is best recommended for automated results.

#### Typical Data

Standard Concentration (pg/ml)	Abs A	Interpolated Concentration	% Interpolated Concentration against Actual Concentration	%Net Deference	Standard Concentration (pg/ml)
0	0.140	--	--	0.000	0
15.6	0.154	14.2	90.8	0.015	15.6
31.25	0.191	28.7	91.7	0.037	31.25
62.5	0.291	65.5	104.8	0.100	62.5
125	0.476	131.1	104.9	0.185	125
250	0.788	241.4	96.5	0.313	250
500	1.489	504.1	100.8	0.701	500
1000	2.594	999.2	99.9	1.105	1000

Typical Graph



#### 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 Detection:** It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus  $2 \times \text{SD}$ . 10 replicates of '0' standards were evaluated and the LOD was found to **15 pg/ml**.

#### Specificity:

The antibodies used in the kit for capture and detection are monoclonal antibodies specific for human IL-5. The standard used in the kit is 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 14 U of 90/586 NIBSC-standard. Please note that the calibration is lot specific.

#### Cross Reactivity:

This assay recognizes natural and recombinant human IL-5. The markers listed below were prepared at 50 pg/ml in Assay Diluent and assayed for cross-reactivity. No significant cross-reactivity or interference was observed.

Recombinant human:

GMCSF      IL-5Ralpha      IL-R

#### Assay Range:

15.6 pg/ml to 1000 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-5 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%

**Reference Values:**

Serum samples from 34 healthy individuals with low CRP levels were evaluated for IL-5 using this ELISA kit. The samples had results in range between 0 to 50 pg/ml. 31 samples obtained value below 17 pg/ml.

**Limitations of Method:**

Any diagnosis should not be based on the results of in-vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis. The KB1067 GENLISA™ Human IL-5 ELISA is a research use kit only and is not licensed for In-Vitro Diagnostic Use.

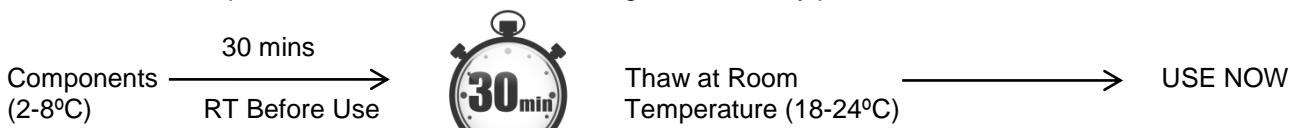
**Safety Precautions:**

- **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.



### SCHEMATIC ASSAY PROCEDURE

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



2. 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 **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 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	Zero Std Zero Std			
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			
3C 4C	Sample			

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## SYMBOLS KEY

	Human IL-5 Microtiter Plate (12X8 wells)
	Human IL-5 Standard lyophilized
	Biotin Conjugated Detection Antibody
	Streptavidin Horseradish Peroxidase
	(1X) Assay Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature