

# **GENLISA™ Human Interleukin 18 (IL-18 / IL18) ELISA**

**REF: KB1082**

Ver 4.4

**RUO**

**ELISA Set for Accurate Quantitation of Human IL-18 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-18 is a cytokine that belongs to the IL-1 superfamily and is produced by macrophages and other cells. The combination of this cytokine and IL12 has been shown to inhibit IL4 dependent IgE and IgG1 production, and enhance IgG2a production in B cells. IL-18 binding protein (IL18BP) can specifically interact with this cytokine, and thus negatively regulate its biological activity.

**Long Name:** Interleukin 18

**Entrez Gene IDs:** 3606 (Human); 16173 (Mouse); 29197 (Rat); 397057 (Porcine); 574151 (Primate)

**Alternate Names:** Iboctadekin; IFN-gamma-inducing factor; IGIF; IGIFIL-1 gamma; IL18; IL-18; IL-18MGC12320; IL-1F4; IL1F4iboctadekin; IL-1g; Interferon gamma-inducing factor; interleukin 18 (interferon-gamma-inducing factor); Interleukin-1 gamma; interleukin-18

**Intended Use:**

GENLISA™ Human Interleukin 18 (IL-18 / IL18) ELISA is specifically designed for the accurate quantitation of human IL-18 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-18 react with already coated affinity purified capture anti-Human IL-18 antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-Human IL-18 is added leading to formation of a sandwich complex of solid phase antibody-Human IL-18-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-18 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-18 present in the samples.

**Materials Provided:**

1. Anti-Human IL-18 Antibody Coated Microtiter Plate (12x8 wells) - 1 no
2. Recombinant Human IL-18 Standard (100 ng/ml, lyophilized) - 2 vials
3. Anti-Human IL-18 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. Microplate Reader able to measure absorbance at 450 nm.
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 and Detection** at 2-8°C. Upon reconstituting, aliquot both standard and detection 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 Assay Diluent (1X) to generate 100 ng/ml. Dilute 7.5 ul of reconstituted Standard with 992.5 ul of Assay Diluent (1X) to generate a 750 pg/ml top standard solution. Perform serial dilutions by using main stock solution as per the below table. Thus the Human IL-18 Standards are 750 pg/ml, 375 pg/ml, 187.5 pg/ml, 93.75 pg/ml, 46.88 pg/ml, 23.44 pg/ml and 11.72 pg/ml. Assay Diluent (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
100 ng/ml	Reconstituted Standard	Lyophilized Standard provided in the Kit + 20 ul of Assay Diluent (1X)
750 pg/ml	Standard No. 8	7.5 ul Reconstituted Standard + 992.5 ul Assay Diluent (1X)
375 pg/ml	Standard No.7	500 ul Standard No.8 + 500 ul Assay Diluent (1X)
187.5 pg/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay Diluent (1X)
93.75 pg/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay Diluent (1X)
46.88 pg/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay Diluent (1X)
23.44 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay Diluent (1X)
11.72 pg/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay Diluent (1X)
0 pg/ml	Standard No.1	Only 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 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 **100 ul** of **diluted Biotinylated Detection Antibody** solution to each well, seal plate and incubate for 2 hours 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 30 minutes 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 30 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:**

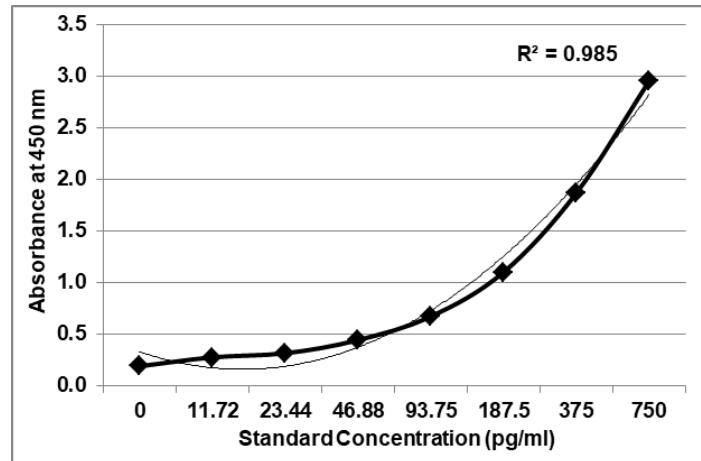
Determine the mean absorbance for each set of duplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight 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 2<sup>nd</sup> 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	0.189	0.196	0.192	--	--
11.72	0.299	0.257	0.278	14.0	119.5
23.44	0.331	0.305	0.318	22.9	97.7
46.88	0.461	0.430	0.445	47.8	102.0
93.75	0.682	0.666	0.674	93.5	99.7
187.5	1.112	1.095	1.103	185.0	98.7
375	1.884	1.855	1.870	377.4	100.6
750	2.867	3.039	2.953	749.2	99.9

**Typical Graph (representative only)**



**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\*SD. 10 replicates of '0' standards were evaluated and the LOD was found to be 10 pg/ml.

**Specificity:**

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

**Cross Reactivity:**

This kit shows cross-reactivity for f recombinant human Pro-IL-18 reads as 29 pg/mL (0.5% cross-reactivity). No significant cross-reactivity or interference was observed.

**Recombinant human:**

IFN- $\gamma$	IL-18 BP $\alpha$ /Fc Chimera	IL-18 RA/Fc Chimera	IL-18 R $\beta$ /Fc Chimera
canine IL-18	canine IL-18	mouse IL-18	porcine IL-18
			rat IL-18

**Assay Range:**

11.72 pg/ml to 750 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-18 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.

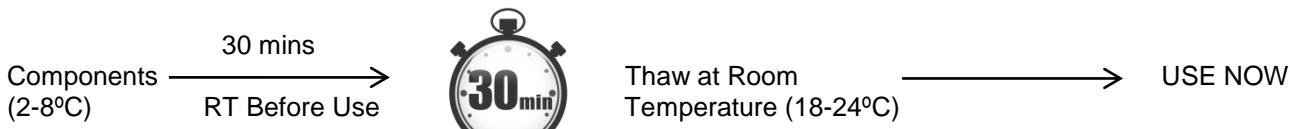
**Safety Precautions:**

- **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/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.



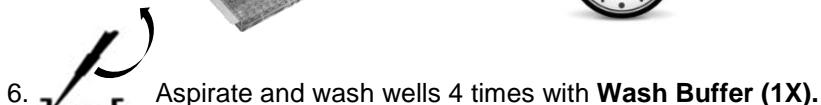
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.



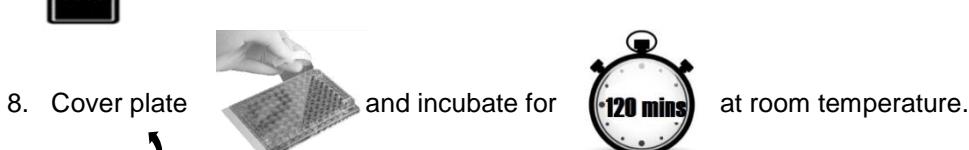
3. Pipette **100 ul Standards** into respective Standard wells.



5. Cover plate and incubate for **120 mins** at room temperature.



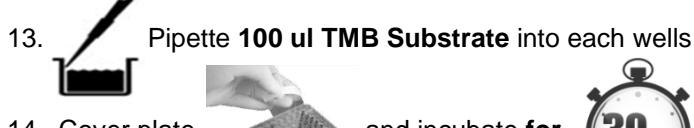
7. Pipette **100 ul diluted Biotinylated Detection Antibody** to all wells.



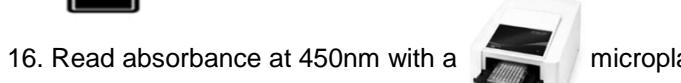
9. Aspirate and wash wells 4 times with **Wash Buffer (1X)**.



11. Cover plate and incubate for **30 min** at room temperature



14. Cover plate and incubate for **30 min** at room temperature.



16. Read absorbance at 450nm with a microplate reader within **30 min** of stopping reaction.

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**SYMBOLS KEY**

	Anti-Human IL18 Coated Microtiter Plate (12x8 wells)
	Recombinant Human IL-18 Standard, Lyophilized
	Anti-Human IL-18 Biotin Conjugated Detection Antibody
	Concentrated Streptavidin Horseradish Peroxidase
	(1X) Assay Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalogue Number
	Expiration Date
	Storage Temperature