GENLISA™ Human Interleukin 8 (IL8) ELISA

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 580 U of 93/730 NIBSC-standard. Please note that the calibration is lot specific.

REF : KB1070

Ver 5.3

RUO

ELISA for Accurate Quantitation of Human IL-8 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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GENLISA™ Human Interleukin 8 (IL8) ELISA

Introduction:

IL-8 is a potent lymphoid cell growth factor that stimulates the growth and survivability of certain B cells and T cells.IL-8 plays a role in host defense, acute phase reactions, immune response, and hematopoiesis. IL-8 is expressed by T cells, B cells, monocytes, fibroblasts, hepatocytes, endothelial cells, and keratinocytes. Recombinant Human IL-8 is a 20.9 kDa protein containing 184 amino acids.

Long Name:
Interleukin 8

Entrez Gene IDs:
3576 (Human); 396880 (Porcine); 403850 (Canine); 493836 (Feline)

Alternate Names:
3-10C; AMCF-I; C-X-C motif chemokine 8; CXCL8; CXCL8SCYB8; Emoctakin; GCP1; GCP-1TSG-1; IL8; IL-8; interleukin 8; K60; LAI; LECT; LUCT; LYNAP; MDNCF; MDNCFb-ENAP; member 8; MONAP; MONAPGCP1; NAF; NAP1; NAP-1NAP1; NCF; Neutrophil-activating protein 1; Protein 3-10C; T cell chemotactic factor; T-cell chemotactic factor; TCF; TSG1

Intended Use:

GENLISA™ Human Interleukin 8 (IL8) ELISA is specifically designed for the accurate quantitation of Human IL-8 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-8 react with already coated affinity purified capture anti-Human IL-8 antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-Human IL-8 is added leading to formation of a sandwich complex of solid phase antibody-Human IL-8-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-8 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-8 present in the samples.

Materials Provided:

1. Anti-Human IL-8 Coated Microtiter Plate (12 x 8 wells) – 1 no.
2. Recombinant Human IL-8 Standard (lyophilized, 0.6 ug/ml) – 2 vials
3. Anti-Human IL-8 Biotin Conjugated Detection Antibody - 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase - 1 vial
5. Streptavidin:HRP Diluent - 12 ml
6. Assay Diluent - 50 ml
7. (20X) Wash Buffer - 25 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. 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.

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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 standard in 20 ul Distilled Water to get a concentration of 0.6 ug/ml. Dilute 5 ul of Reconstituted standard with 2995 ul of Assay Diluent to prepare 3ml of the top standard (1000 pg/ml). Perform serial dilutions by using main stock solution as per the below table. Thus the Human IL-8 Standards concentration are 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml and 15.62 pg/ml. Assay Diluent serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
0.6 ug/ml	Reconstituted Standard	Lyophilized Standard provided in the Kit + 20 ul Distilled water
1000 pg/ml	Standard No.7	5 ul Reconstituted Standard + 2995 ul Assay Diluent
500 pg/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay Diluent
250 pg/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay Diluent
125 pg/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay Diluent
62.5 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay Diluent
31.25 pg/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay Diluent
15.62 pg/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay Diluent
0 pg/ml	Standard No.0	Only Assay diluent

3. Add 100 ul of prepared **Standards** and **Samples** to the respective wells.

4. Add 50 ul of **diluted Biotinylated Detection Antibody** solution to each well. Mix well.

5. Seal plate and Incubate for 2 hours at 37°C.

6. 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.

7. Add 100 ul of diluted **Streptavidin: HRP** solution to each well.

8. Seal plate and incubate for 30 minutes at 37°C.

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

10. 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.

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

12. 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.

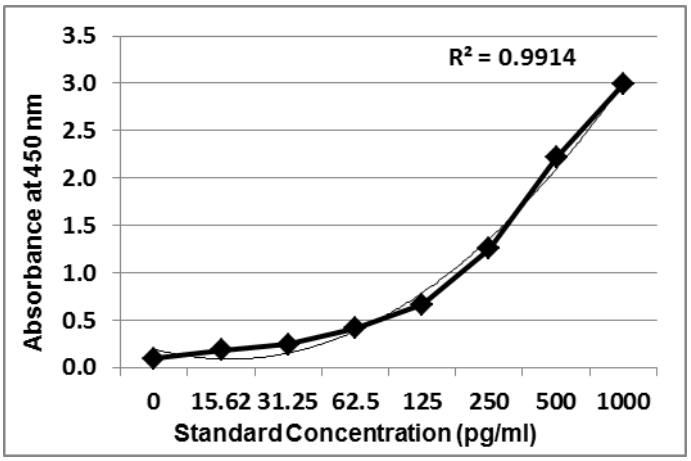
Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown cytokine concentrations, find the unknowns mean absorbance value on the y-axis and draw a horizontal 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 4-PL (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	% STD Deviation	CV	%CV
0	0.096	0.097	0.096	--	--	0.1	0.01	0.7
15.62	0.186	0.178	0.182	18.4	117.8	0.6	0.03	3.2
31.25	0.236	0.248	0.242	35.0	112.1	0.9	0.04	3.6
62.5	0.426	0.401	0.414	73.3	117.3	1.8	0.04	4.3
125	0.640	0.682	0.661	122.0	97.6	3.0	0.04	4.5
250	1.287	1.213	1.250	240.7	96.3	5.2	0.04	4.2
500	2.159	2.273	2.216	515.4	103.1	8.0	0.04	3.6
1000	3.069	2.917	2.993	986.8	98.7	10.7	0.04	3.6

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 **15 pg/ml.**

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Human IL-8. 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 580 U of 93/730 NIBSC-standard. Please note that the calibration is lot specific.

Cross-Reactivity:
This assay recognizes natural and recombinant human IL-8. The markers listed below were prepared at 250 pg/ml in Assay Diluent and assayed for cross-reactivity. No significant cross-reactivity or interference was observed.

Recombinant human:

ANG	AR	CNTF	β-ECGF	EGF	Epo	FGF acidic	FGF basic	FGF-4
FGF-5	FGF-6	G-CSF	GM-CSF	GROα	GROβ	GROγ	gp130	HB-EGF
HGF	I-309	IFN-γ	IGF-I	IGF-II	IL-1α	IL-1β	IL-1ra	IL-1 RI
IL-1 RII	IL-2	IL-2 Rα	IL-3	IL-3 Rα	IL-4 R	IL-5	IL-5 Rβ	IL-6
IL-6 R	IL-7	IL-9	IL-10	IL-11	IL-12	IL-13	IP-10	KGF
LAP (TGF-β1)	LIF	M-CSF	MCP-1	MCP-2	MCP-3	MIP-1α	MIP-1β	β-NGF
OSM	PD-ECGF		PDGF-AA	PDGF-AB		PDGF-BB	PF-4	PTN
RANTES	SCF	SLPI	TGF-α	TGF-β1		TGF-β3	TGF-β RII	TNF-α
TNF-β	TNF RI	TNF RII	VEGF					

Recombinant mouse:
GM-CSF IL-1α IL-1β IL-3 IL-4 IL-5 IL-5 Rα IL-6 IL-7 IL-9 IL-10 IL-13 KC LIF MIP-1α MIP-1β SCF TNF-α

Recombinant amphibian:
TGF-β5

Natural proteins:
bovine FGF acidic bovine FGF basic human PDGF porcine PDGF human TGF-β1 porcine TGF-β1 porcine TGF-β2

Assay Range:
15.62 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-8 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%

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.

Cited References:

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The biochemical role of differentclasses of ytokine in cancer. E Simionică, O Gheorghiță - Scientific Collection" InterConf", 2021 - ibn.idsi.md.… Interleukin 8 values were measured using the Sandwich ELISA technique using the Human IL-8 ELISA kit produced by Krishgen BioSystem in Spain, in the presence of standard …

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… Cytokines were quantified by specific Human ELISA kit sets (Krishgen Biosystems) following the manufacturer's instructions. In this study, authors have measured the concentration of

SCHEMATIC ASSAY PROCEDURE

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

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

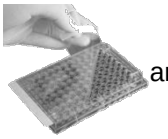
Thaw at Room Temperature (18-24ºC) → USE NOW

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

Kit Components → COOL (2-8ºC) AVOID → THAW (22±4ºC) →  → THAW (22±4ºC) AVOID → COOL (2-8ºC) → NO CHANGE IN RESULTS

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

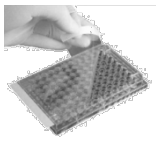
4. Pipette **50 ul** diluted **Biotinylated Detection antibody** to all wells. Mix well.

5. Cover plate  and incubate for 

at 37°C.

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

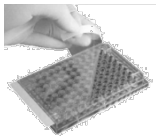
7. Pipette **100 ul** diluted **Streptavidin:HRP** to all wells.

8. Cover plate  and incubate for 

at 37°C.

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

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

11. Cover plate  and incubate for 

at 37°C.

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

13 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 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	Anti-Human IL-8 Coated Microtiter Plate (12x8 wells)
STD	Recombinant Human IL-8 Standard , Lyophilized
BIO CONJ	Anti-Human IL-8 Biotin Conjugated Detection Antibody
STRP HRP	Concentrated Streptavidin Horseradish Peroxidase
STRP HRP DIL	Streptavidin:HRP Diluent
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