ELISA VALIDATION GUIDE

CYTOKINE ASSAYS FOR USE IN

DRUG DISCOVERY RESEARCH,
BIOPHARMA AND

CELL & GENE THERAPY
APPLICATIONS

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

VALIDATION OF PRECISIONBIND HUMAN IL-8 ELISA KIT (Catalog No KB1070) AS PER FDA/ICH GUIDELINES FOR BIOANALYTICAL METHOD VALIDATION

This validation protocol has been adopted in line with the Methodology and Analytical Procedures Guideline recommended by FDA/ICH.

Document History

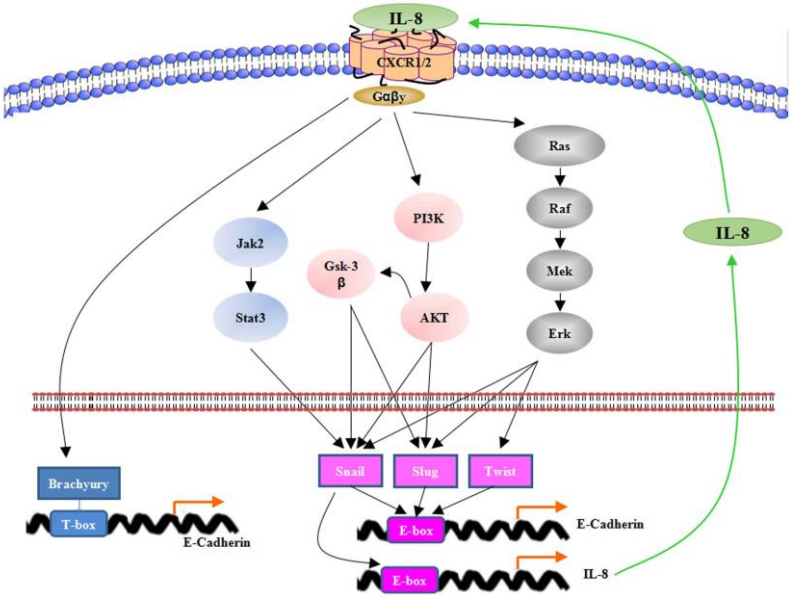
First Codification	History	Date
Version#1	**VALIDATION DATA OF PRECISIONBIND HUMAN IL-8 ELISA KIT (Catalog No KB1070)**	31.07.2025

Approved Quality Control	Approved Product Development	Approved Operations Head
Prairna B	Atul G	K Jain

Background

1. Introduction to Interleukin-8 (IL-8)

Interleukin-8 (IL-8), also known as CXCL8, is a chemokine primarily produced by monocytes, macrophages, endothelial cells, and epithelial cells in response to inflammatory stimuli. IL-8 plays a significant role in neutrophil chemotaxis and activation, contributing to the innate immune response. It also triggers angiogenesis, tumor progression, and metastasis in various cancers. Aberrant IL-8 expression is associated with chronic inflammation, autoimmune disorders, and malignancies, making it a significant biomarker and therapeutic target in drug discovery and clinical research.



2. Significance in Drug Discovery Research

2.1 Target Identification and Validation

- IL-8 is central to neutrophil-mediated inflammation and tissue remodeling.
- Elevated IL-8 levels are observed in chronic obstructive pulmonary disease (COPD), asthma, psoriasis, rheumatoid arthritis, and several cancers (e.g., breast, prostate, colorectal).
- Targeting IL-8 or its receptors (CXCR1 and CXCR2) is a validated strategy in anti-inflammatory and anti-cancer therapies.

2.2 Assay Development

- IL-8 serves as a model chemokine for screening drugs targeting inflammatory and metastatic pathways:
    - ELISA or multiplex assays for IL-8 quantification in serum, plasma, or cell culture supernatants
    - Cell-based chemotaxis assays using neutrophils or CXCR1/2-expressing cells
    - Reporter gene assays for CXCR1/CXCR2 signaling modulation

2.3 Biomarker for Efficacy and Safety

- IL-8 levels correlate with drug-induced inflammation, angiogenesis inhibition, or tumor regression.
- Used in oncology and pulmonology trials as a pharmacodynamics and prognostic biomarker.
- Monitoring IL-8 helps assess immune activation and potential cytokine-related toxicities in early-stage clinical evaluations.

3. Relevance in Biopharmaceutical Development

3.1 Monoclonal Antibodies and Biologics

- Anti-IL-8 and anti-CXCR1/2 monoclonal antibodies (e.g., HuMax-IL8, Reparixin) are under investigation for cancer, COVID-19-related inflammation, and autoimmune conditions.
- Biologic development involves:
  - Ligand-receptor binding assays (e.g., IL-8–CXCR1/CXCR2 interaction)
  - Inhibition potency assays (e.g., chemotaxis or calcium flux)
  - Comparability and stability studies for regulatory filings and biosimilar candidates

3.2 PK/PD Studies

- IL-8 levels are monitored in clinical trials as pharmacodynamics endpoints, particularly in therapies targeting neutrophil recruitment or angiogenesis.
- PK/PD correlations help optimize dose regimens and evaluate therapeutic efficacy.

3.3 Safety Evaluation

- IL-8 suppression can impair host defense against infections, especially in pulmonary and cutaneous tissues.
- Monitoring is critical in biologic therapies to avoid neutropenia-related complications and delayed tissue repair.4. Importance in Cell and Gene Therapy (CGT).

4. Importance in Cell and Gene Therapy (CGT)

4.1 Cytokine Release Syndrome (CRS) Monitoring

- IL-8 is a secondary but relevant mediator in CRS during CAR-T and gene-modified T cell therapies.
- Elevated IL-8 levels, in combination with IL-6 and TNF-α, signal hyper inflammatory states and guide clinical management of CRS.

4.2 Immune Modulation Biomarker

- IL-8 quantification helps profile innate immune cell recruitment and tumor microenvironment remodeling during CGT trials.
- Plays a role in assessing myeloid-derived suppressor cell (MDSC) dynamics and immune escape mechanisms in oncology-based CGT.

4.3 Genetic Modulation

- Gene-editing tools are being explored to:
    - Suppress IL-8 expression in engineered immune cells to reduce off-target inflammation
    - Modify CXCR1/2 expression to reprogram T cell or NK cell trafficking
    - Improve tumor homing and anti-tumor activity of adoptive cell therapies

Scope of Validation

The PrecisionBind Human IL-8 (Catalog No KB1070) kit is considered by us during the validation of this kit in accordance with ICH Q2 (R1) guidelines. The document is prepared based on tests run in our laboratory and does not necessarily seek to cover the testing that may be required at user's end for registration in, or regulatory submissions. The objective of this validation is to demonstrate that it is suitable for its intended purpose - detection of IL-8

Validation characteristics considered by us in accordance with the guidelines are listed below:

- Specificity and Selectivity.
- Sensitivity (LOD & LOQ).
- Linearity and Range.
- Accuracy and Precision (Intra/Inter-Assay).
- Matrix Effect (serum, plasma and CSF).
- Sample Handling and Storage Conditions.
- References (IL-8 Beta Cmax Values and Recommended ELISA Range).

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

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 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.

For any queries or support on the data and its performance, please contact us at sales1@krishgen.com

Intended Use of the ELISA

The PrecisionBind Human Interleukin 8 (IL-8) ELISA kit is intended to measure the IL-8 (Interleukin 8) in serum, plasma, cell culture supernatant and other biological fluids.

Principle of the Assay

This ELISA is a sandwich immunoassay. Antibodies are coated on 96 well plates. The antigen protein present in sample and standard respectively bind to the coated wells. The wells are washed and an antibody:HRP Conjugate is added which binds to the bound complex in the well. Washing is performed to remove any unbound material. TMB substrate is added and the enzyme reaction is stopped by dispensing of stop solution into the wells. The optical density (OD) of the solution at 450 nm is directly proportional to the amount of antigen protein present in the standard or samples.

Validation Parameters and Acceptance Criteria

1. IL-8 Cmax Values and Recommended ELISA Range

This table summarizes IL-8 Cmax levels across diseases and suggests corresponding ELISA working ranges.

Application	Expected IL-8 Range (pg/mL)	Recommended ELISA Range (pg/mL)
Healthy baseline	<15	0–100
Chronic inflammatory disease (RA, IBD, psoriasis)	50-500 (peaks up to 1000)	0–1,500
Sepsis / cytokine storm	500–35000 (rare >10,000)	0–10,000
Cell & gene therapy cytokine release monitoring	100–2,000	0–5,000

Note: Assay sensitivity <5 pg/mL recommended for baseline detection; upper limit ≥5,000 pg/mL advised for CRS monitoring

The PrecisionBind Human IL-8 ELISA kit is developed using an assay range of 7.8 - 500 pg/ml with the dilutional linearity accuracy to measure responses as per the application table above on patient Cmax values. The kit has also been validated upto 1600 fold dilution and the values are within the acceptable range.

2. Specificity and Selectivity

2.1 Specificity

The capture antibody and detection antibody are both specific to IL-8 and are monoclonal antibodies. They show a high affinity to bind to native as well as recombinant IL-8.

2.2 Selectivity

The ELISA has no or low cross reactivity to IL-1β (IL-1beta), IL-6, or TNF-β (TNFbeta).

2.3 NIBSC validation

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 580 U of 93/730 NIBSC-standard.
Therefore 1000 pg/ml is equivalent to 580 U of IL-8 as per NIBSC.

2.4 LOD, LOQ and IC50

LOD (Limit of Detection)
The lowest analyte concentration that can be reliably distinguished from blank/background noise but not necessarily quantified precisely.

Statistically:
LOD = Mean of Blank + 3X SD of Blank
(3σ criterion is most common).

LOD for PrecisionBind Human IL-8 ELISA = 0.2 pg/ml

LOQ (Limit of Quantitation)
The lowest analyte concentration that can be quantified with acceptable accuracy and precision.

Statistically:
LOQ = Mean of Blank + 10X SD of Blank
(10σ criterion is most common).

LOQ for PrecisionBind Human IL-8 ELISA – 0.6 pg/ml

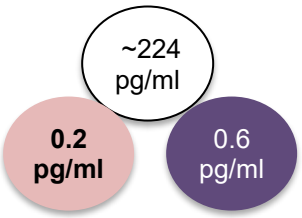
IC50 in ELISA (Half Maximal Inhibitory Concentration)
IC50 = The concentration of an inhibitor (drug, antibody, compound) required to reduce the signal (e.g., binding, enzymatic activity) by 50% compared to the maximum signal in the assay.

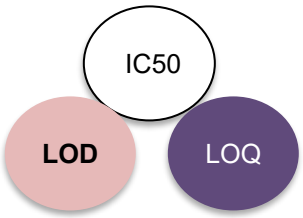
In ELISA, this is commonly used for:
Neutralization ELISA: Quantifies potency of antibodies inhibiting target–ligand interaction.
Drug Potency Testing: Measures concentration at which drug inhibits 50% of target activity.

IC50 for PrecisionBind Human IL-8 ELISA = ~224 pg/ml

Summary:

Parameter	Value (pg/mL)
LOD	0.2 pg/ml
LOQ	0.6 pg/ml
IC50	~ 224 pg/ml

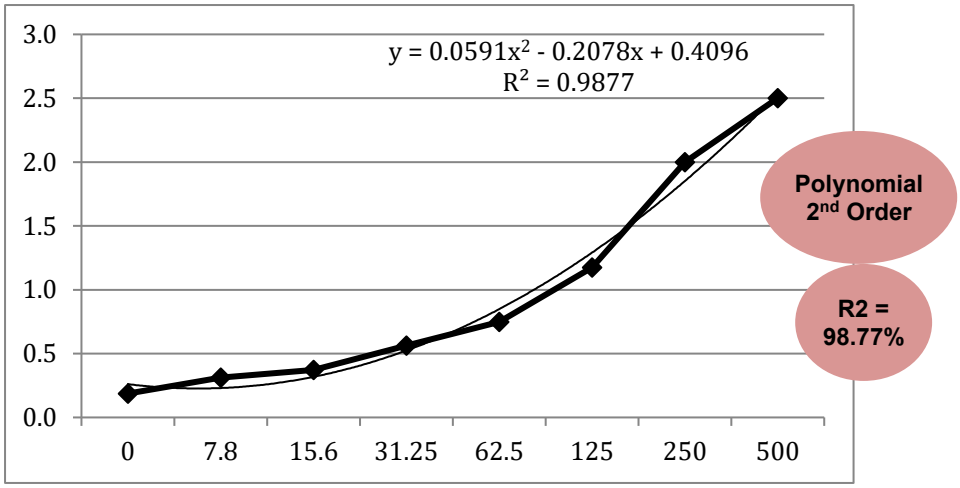


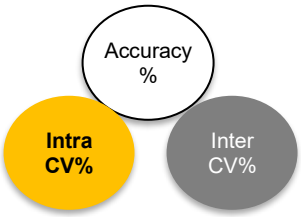


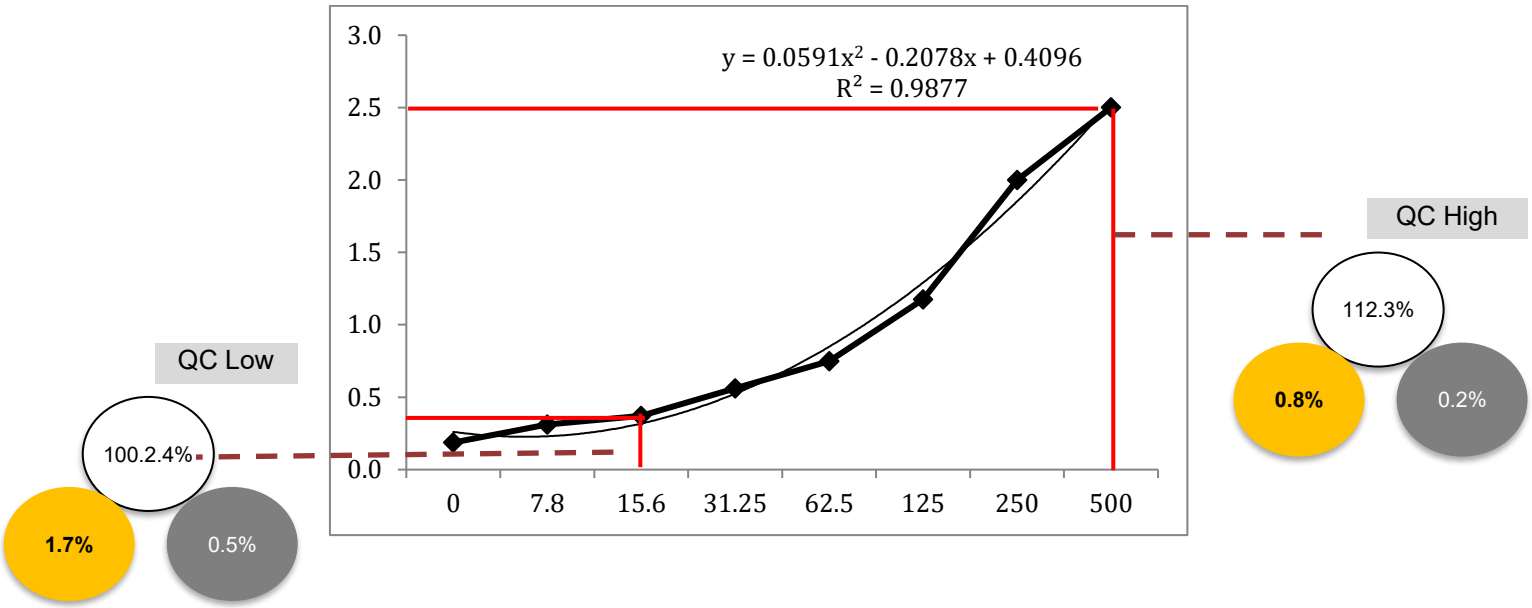
Regulatory Note:
LOD S/N ≥ 3:1, LOQ ≥ 10:1, %CV ≤ 20% **S/N = Signal / Noise Ratio*

3. Linearity and Range

Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery
0	0.187	--	--
7.8	0.311	9.4	120.5
15.6	0.371	17.7	113.7
31.25	0.562	39.2	125.4
62.5	0.749	60.0	96.0
125	1.175	112.8	90.3
250	2.000	273.4	109.4
500	2.501	481.7	96.3
Positive Control (250 pg/ml)	1.743	255.1	102.0
Low QC control (15.6 pg/ml)	0.378	15.6	100.2
High QC control (400 pg/ml)	2.632	449.3	112.3







4. Accuracy and Precision (Intra/Inter-Assay)

A) Intra-assay:

Standard (pg/ml)	Mean OD450	SD	%CV
7.8	0.382	0.52	1.7
125	1.183	2.84	2.9
500	2.496	1.64	0.8

B) Inter assay:

Standard (pg/ml)	Mean OD450	SD	%CV
7.8	0.384	0.15	0.5
125	1.188	0.34	0.4
500	2.492	0.39	0.2

4. Parallelism and Matrix Effect

Sample Dilution factor – Human Serum, Human Plasma and Human CSF samples have been tested. Sample dilution Factor for all three matrices is 1:50 dilution.

Neat Human Serum, Human Plasma and Human CSF were spiked with 250 pg/ml Human IL-8 and ELISA assay was run.

Sample	Mean OD450	Interpolated Concentration	% Recovery
Neat CSF samples	2.971	528.2	Out of range
Neat Plasma	3.175	576.9	230.8
Neat Human Serum	3.818	735.1	294.0

A) Serum:

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:100 dilution	500	2.352	436.9	87.4	114.4
1:200 dilution	250	1.598	246.5	98.6	101.4
1:400 dilution	125	0.913	100.6	80.5	124.3
1:800 dilution	62.5	0.731	58.2	93.1	107.4
1:1600 dilution	31.25	0.555	28.7	91.8	108.9
1:3200 dilution	15.6	0.278	4.8	30.7	325.8
1:6400 dilution	7.8	0.200	--	0.0	--

B) Plasma:

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:100 dilution	500	2.569	434.9	87.0	115.0
1:200 dilution	250	1.652	236.5	94.6	105.7
1:400 dilution	125	1.156	140.1	112.1	89.2
1:800 dilution	62.5	0.810	79.3	126.9	78.8
1:1600 dilution	31.25	0.567	41.3	132.0	75.8
1:3200 dilution	15.6	0.313	8.1	52.0	192.5
1:6400 dilution	7.8	0.241	1.3	16.2	616.8

C) Cerebrospinal Fluid (CSF):

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:100 dilution	500	2.610	444.3	88.9	112.5
1:200 dilution	250	1.674	240.9	96.4	103.8
1:400 dilution	125	1.178	144.1	115.3	86.7
1:800 dilution	62.5	0.832	83.0	132.8	75.3
1:1600 dilution	31.25	0.590	44.6	142.9	70.0
1:3200 dilution	15.6	0.343	11.5	73.5	136.2
1:6400 dilution	7.8	0.268	3.6	45.8	218.8

Results:

i) Parallelism is maintained across the 1:100 to 1:1600 dilutions.
ii) % Recovery for most dilutions falls within the acceptable range of 80%–120%.
iii) No significant matrix effect observed at higher dilutions.
iv) The PrecisionBind Human IL-8 ELISA kit was tested for matrix effect on human serum, plasma, CSF and physiological buffer 7.4 to mimic tear fluid samples.

6. Sample Handling and Storage Conditions

A) Sample 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.

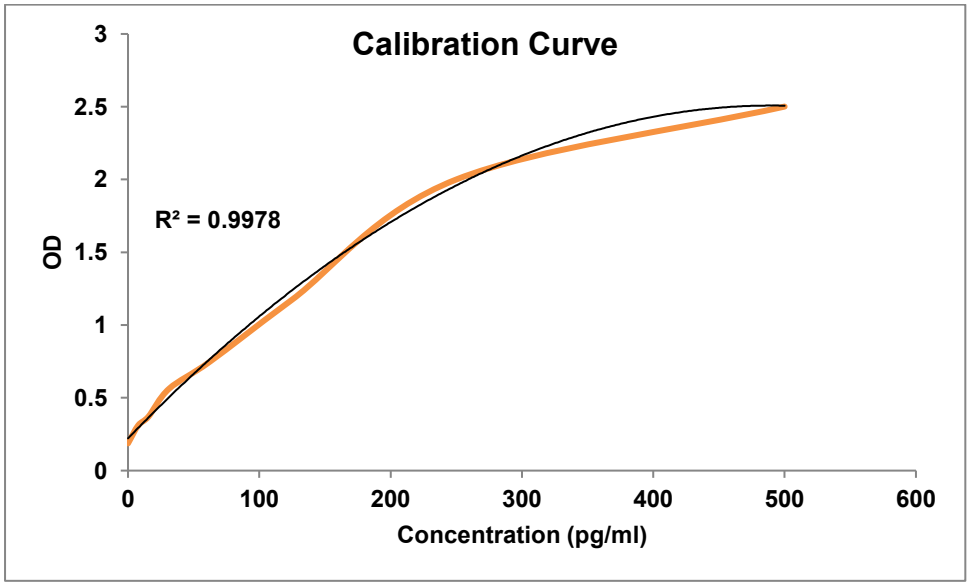
Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

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.

B) Storage conditions:

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

Graphs, Maps and Appendices:



Calibration of Standard Used in the KIT

NIBSC Standard 93/730 IL-8

PrecisionBind Human IL-8 Kit Standard

Matrix Effect Heat Map

	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400
Serum							
Plasma							
Cerebrospinal fluid (CSF)							

Determined Limits for Acceptance according to EMA/FDA and CLSI regulations

	Limits for Acceptance (EMA/FDA)	Determined Limits for Acceptance (CLSI)
Intra Precision	CV < 20% (25% at LLOQ)	-
Inter Precision	CV < 20 % (25% at LLOQ)	-
Accuracy at LLOQ	Recovery 100 ± 20% (100 ±25%	-
Total Error (TE)	TE < 30% (40% at LLOQ and ULOQ)	-
Specificity/Interference	Recovery 100 ± 25%2	H (null hypothesis) = 100 ± 25 %
Parallelism/Linearity	CV < 30%2	Deviation from linearity < 20%
LLOQ / LoQ	Recovery 100 ± 25%	TE % < 32.9%

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Version No. 1.0, dated 31.07.2025