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-1 BETA (Catalog No KB1063) 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

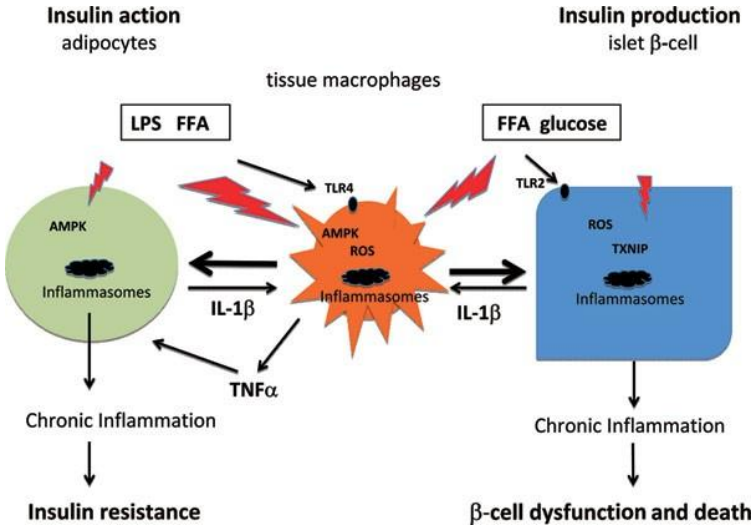
First Codification	History	Date
Version#1	**VALIDATION DATA OF PRECISIONBIND HUMAN IL-1 BETA ELISA KIT (Catalog No KB1063)**	31.07.2025

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

Background

1. Introduction to IL-1β

Interleukin-1 beta (IL-1β) is a potent pro-inflammatory cytokine majorly developed by activated monocytes, dendritic cells, macrophages and epithelial cells in response to any type of infection or injury. It has a significant role in the innate immune response, fever induction, recruitment of leukocytes, and activation of inflammasome. Dysregulated IL-1β expression is highly associated with a vast range of conditions including autoimmune diseases, metabolic disorders, cardiovascular disease, and cancer. As a result of this, IL-1β is considered a vital biomarker and therapeutic target in modern drug discovery programs.



2. Significance in Drug Discovery Research

2.1 Target Identification and Validation

- IL-1β is the key regulator of inflammation, which acts upstream of cytokine cascades and activates the NF-κB and MAPK pathways.
- In rheumatoid arthritis elevated IL-1β is observed along with in gout, multiple sclerosis, type 2 diabetes, and atherosclerosis.
- By Targeting IL-1β signalling (e.g., IL-1R1 blockade or inflammasome inhibition), it has developed as an established therapeutic strategy.

2.2 Assay Development

- IL-1β is widely used in in vitro and in vivo models for screening immunomodulatory compounds:
  - ELISA and ultrasensitive immunoassays for quantification in serum, plasma, or cell culture supernatants
  - Inflammasome activation assays (e.g., NLRP3 pathway studies)
  - Cell-based functional assays (e.g., THP-1 macrophage stimulation models)

2.3 Biomarker for Efficacy and Safety

- IL-1β potentially acts as a pharmacodynamic marker in clinical trials for its role as anti-inflammatory and immunosuppressive agents.
- Frequently measured in cytokine panels to assess immune activation or risk of cytokine release syndrome (CRS).

3. Relevance in Biopharmaceutical Development

3.1 Monoclonal Antibodies and Biosimilars

- IL-1β-neutralizing agents (e.g., Canakinumab, Anakinra, Rilonacept) have shown its efficacy in autoinflammatory syndromes and chronic inflammatory conditions.
- Biosimilar and novel therapeutic development involves:
  - L-1β binding affinity assays
  - Neutralization assays using stimulated immune cells
  - Comparability and potency assessments under regulatory frameworks

3.2 PK/PD Studies

- IL-1β levels are highly considered as a biomarker for inflammation and target engagement involved with clinical trials, especially for anti-cytokine therapies and NLRP3 inhibitors.

3.3 Safety Evaluation

- Monitoring the level of IL-1β is critical to ensure controlled immunomodulation, as over suppression can affect host defence against infections.

4. Importance in Cell and Gene Therapy (CGT)

4.1 Cytokine Release Syndrome (CRS) Monitoring

- IL-1β is a core cytokine in CRS associated with CAR-T cell and TCR-engineered therapies.
- Real-time IL-1β measurement can detect early intervention strategies (e.g., Anakinra co-administration).

4.2 Immune Modulation Biomarker

- IL-1β is critical in monitoring activation of innate immune system and serves as a primary readout in preclinical and clinical CGT studies.

4.3 Genetic Modulation

- Gene-editing strategies are being explored to investigate the knockdown of IL-1β or inflammasome components to decrease inflammation in diseases like CAPS or improve cell therapy tolerability.

Scope of Validation

PrecisionBind Human IL-1 Beta (Catalog No KB1063) 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 Human Interleukin 1-Beta.
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-1 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

PrecisionBind Human Interleukin 1 Beta (IL-1B / IL1b) ELISA kit is intended to measure the IL-1B (Interleukin 1 Beta) 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-1B Cmax Values and Recommended ELISA Range

This table summarizes IL-1B Cmax levels across diseases and suggests corresponding ELISA working ranges.

Application	Expected IL-1B Range (pg/ml)	Recommended ELISA Range (pg/ml)
Healthy Baseline	<5	0 - 25
Chronic Inflammatory Disease (RA, IBD, Psoriasis)	10 - 150 (peaks up to 300)	0 - 1,000
Sepsis / Cytokine Storm	100 - 2,000 (rare >5000)	0 - 2000
Cell & Gene Therapy Cytokine Release Monitoring	20-800	0–2,000

Note: Assay sensitivity < 2 pg/mL recommended for baseline detection; upper limit ≥ 3,000 pg/ml advised for CRS monitoring.

The PrecisionBind Human IL-1B ELISA kit is developed using an assay range of 3.9 - 250 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 1280 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-1B and are monoclonal antibodies. They show a high affinity to bind to native as well as recombinant IL-1B.

2.2 Selectivity

The ELISA has no or low cross reactivity to IL-1alpha.

2.3 NIBSC validation

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 165 U of 86/680 NIBSC-standard.
Therefore 1000 pg/ml is equivalent to 165 U of IL-1B 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-1B ELISA = 0.1 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-1B ELISA – 0.3 pg/ml

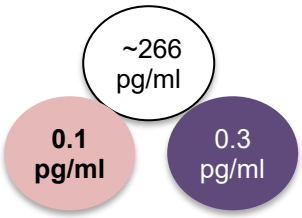
IC50 in ELISA (Half Maximal Inhibitory Concentration)
IC50 = The concentration of an inhibitor (drug, antibody, compound) required to reduce the signal (e.g., binding, enzymatic activity) by 50% compared to the maximum signal in the assay.

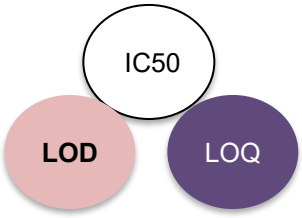
In ELISA, this is commonly used for:
Neutralization ELISA: Quantifies potency of antibodies inhibiting target–ligand interaction.
Drug Potency Testing: Measures concentration at which drug inhibits 50% of target activity.

IC50 for PrecisionBind Human IL-1B ELISA = ~266 pg/ml

Summary:

Parameter	Value (pg/mL)
LOD	0.1 pg/ml
LOQ	0.3 pg/ml
IC50	~ 266 pg/ml

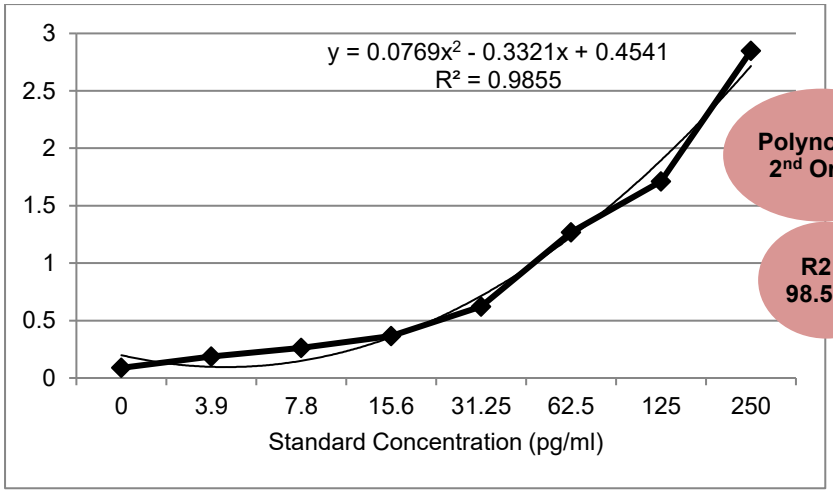


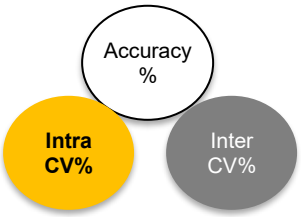


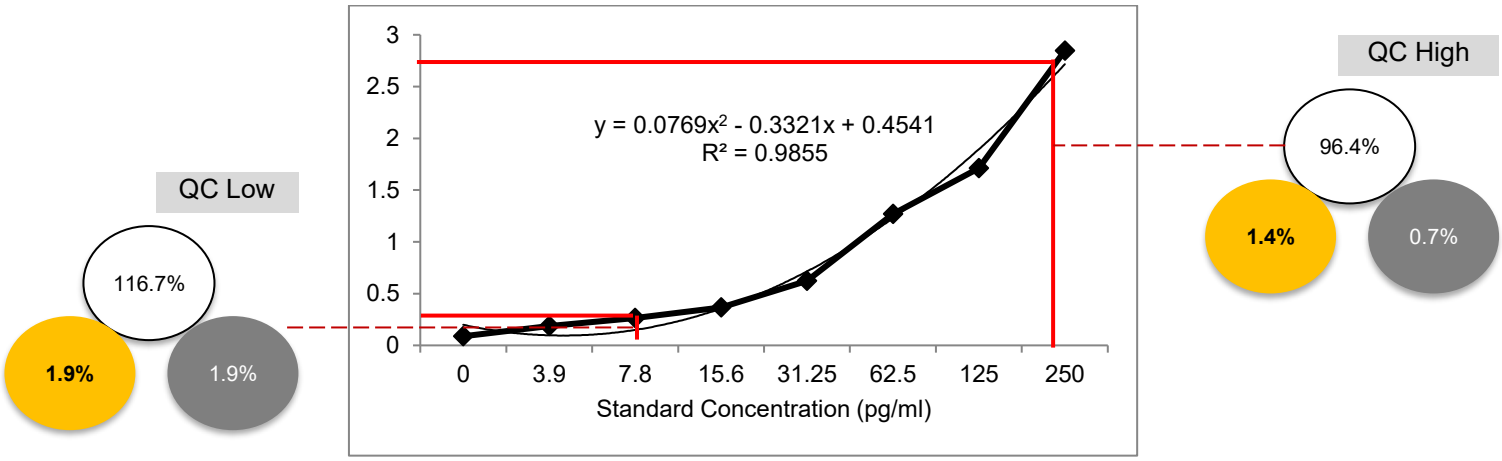
Regulatory Note:
LOD S/N ≥ 3:1, LOQ ≥ 10:1, %CV ≤ 20% **S/N = Signal / Noise Ratio*

3. Linearity and Range

Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery
0	0.088	0.4	--
3.9	0.188	4.3	109.2
7.8	0.264	7.7	99.3
15.6	0.365	12.9	82.4
31.25	0.623	27.8	89.1
62.5	1.269	75.2	120.3
125	1.712	115.4	92.4
250	2.847	252.8	101.1
Positive Control (200 pg/ml)	2.258	202.0	101.0
Low QC control (7.8 pg/ml)	0.219	9.1	116.7
High QC control (250 pg/ml)	2.829	240.9	96.4







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

A) Intra-assay:

Standard (pg/ml)	Mean OD450	SD	%CV
3.9	0.173	0.21	1.9
62.5	1.225	2.93	2.9
250.0	2.792	3.20	1.4

B.) Inter assay:

Standard (pg/ml)	Mean OD450	SD	%CV
3.9	0.169	0.32	1.9
62.5	1.237	1.45	1.2
250.0	2.805	1.90	0.7

5. 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 1:10 dilution.

Neat Human Serum, Human Plasma and Human CSF were spiked with 125 pg/ml Human IL1 beta and ELISA assay was run.

Sample	Mean Mean OD450	Interpolated Concentration	% Recovery
Neat Human CSF	2.800	265.8	212.6
Neat Human Plasma	2.775	261.7	209.3
Neat Human Serum	2.909	284.4	227.5

A) Serum:

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:20 dilution	250	3.118	251.2	100.5	99.5
1:40 dilution	125	2.067	121.2	96.9	103.2
1:80 dilution	62.5	1.420	68.1	109.0	91.7
1:160 dilution	31.25	0.765	28.1	89.9	111.2
1:320 dilution	15.6	0.483	14.4	92.3	108.5
1:640 dilution	7.8	0.387	10.2	131.1	76.4
1:1280 dilution	3.9	0.205	3.1	80.0	125.3

B) Plasma:

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:20 dilution	250	2.498	229.2	91.7	109.1
1:40 dilution	125	1.507	114.2	91.4	109.4
1:80 dilution	62.5	0.912	64.3	102.9	97.2
1:160 dilution	31.25	0.573	38.4	122.8	81.4
1:320 dilution	15.6	0.299	16.8	107.7	93.0
1:640 dilution	7.8	0.201	8.0	102.3	97.9
1:1280 dilution	3.9	0.172	5.0	127.2	78.8

C) Cerebrospinal Fluid (CSF):

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:20 dilution	250	2.528	233.7	93.5	107.0
1:40 dilution	125	1.492	112.9	90.3	110.7
1:80 dilution	62.5	0.889	62.5	100.1	99.9
1:160 dilution	31.25	0.601	40.5	129.7	77.1
1:320 dilution	15.6	0.304	17.2	110.4	90.8
1:640 dilution	7.8	0.193	7.2	92.0	108.9
1:1280 dilution	3.9	0.168	4.5	115.7	86.6

Results:

i. Parallelism is generally maintained across the 1:20 to 1:1280 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 IL1 beta 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.

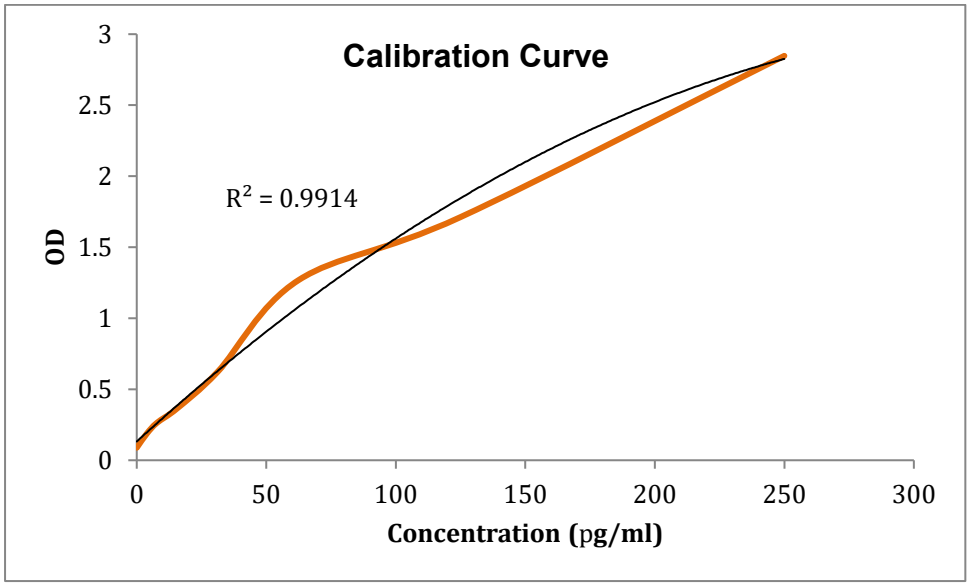
Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

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 86/680 IL1-Beta
- PrecisionBind Human IL1-Beta Kit Standard

Matrix Effect Heat Map

	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
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%

References

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Version 1.0, dated 31.07.2025

