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-10 ELISA KIT (Catalog No KB1072) 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

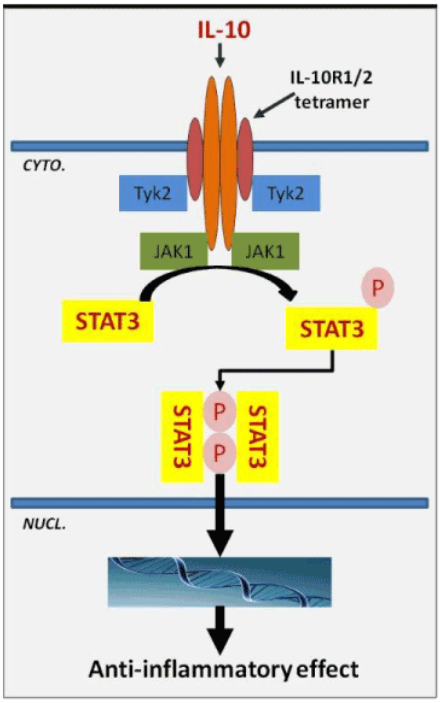
First Codification	History	Date
Version#1	**VALIDATION DATA OF PRECISIONBIND HUMAN IL-10 ELISA KIT (Catalog No KB1072)**	31.07.2025

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

Background

1. Introduction to IL-10

Interleukin-10 (IL-10) is a type of anti-inflammatory cytokine secreted by monocytes, regulatory T cells (Tregs), B cells, and certain subsets of dendritic cells. It has a significant immunoregulatory role by inhibiting the expression of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6), MHC class II molecules, and co-stimulatory molecules on antigen-presenting cells (APCs). IL-10 is important for preventing excessive immune activation and maintenance of tissue homeostasis. Dysregulation of IL-10 signalling cascade is very much associated with autoimmune disorders, chronic infections, cancer, and transplant rejection. Due to its presence of immunosuppressive properties, IL-10 is regarded as a promising therapeutic target and biomarker in inflammatory, infectious, and oncological drug development.



2. Significance in Drug Discovery Research

2.1 Target Identification and Validation

- IL-10 is at the core of anti-inflammatory and immunosuppressive signalling pathways (e.g., STAT3, SOCS3).
- Reduced expression of IL-10 is involved with autoimmune diseases such as inflammatory bowel disease (IBD), lupus, and multiple sclerosis.
- Therapeutic strategies aim to increase IL-10 activity or mimic its signalling to control immune-mediated pathologies.

2.2 Assay Development

- IL-10 is considered as the primary marker in evaluating the efficacy of anti-inflammatory agents and immunomodulators:
  - ELISA for quantification of IL-10 in serum, plasma, or cell culture supernatants.
  - qPCR and flow cytometry for assessing IL-10 gene and protein expression in immune cells.
  - Multiplex assays for cytokine profiling alongside IL-6, IL-1β, and TNF-α.

2.3 Biomarker for Efficacy and Safety

- IL-10 levels indicate the anti-inflammatory status and resolution of inflammation during therapy.
- It plays a significant role as a protective biomarker in cytokine-driven pathologies and the activity is monitored to evaluate balance in immune modulation during preclinical and clinical studies.

3. Relevance in Biopharmaceutical Development

3.1 Monoclonal Antibodies and Biosimilars

- Recombinant IL-10 and IL-10 fusion proteins (e.g., PEGylated IL-10, AM0010) are being extensively studied for autoimmune diseases, cancer immunotherapy, and fibrosis.
- Biopharmaceutical development includes:
  - IL-10 receptor binding assays.
  - Functional activity assays (e.g., suppression of inflammatory cytokines in co-culture systems).
  - Stability and comparability studies for formulation and regulatory approval

3.2 PK/PD Studies

- IL-10 levels are often considered as pharmacodynamic (PD) markers in trials of anti-inflammatory biologics and engineered cytokines.
- It is used for development ofd correlate dose-dependent immunosuppression and anti-tumor activity in cytokine-based therapies.

3.3 Safety Evaluation

- Prolonged exposure to IL-10 can cause immunosuppression which increases the chances of susceptibility to chronic infections or tumor progression.
- Therefore, tight control of IL-10 expression or delivery is essential in therapeutic development.

4. Importance in Cell and Gene Therapy (CGT)

4.1 Cytokine Release Syndrome (CRS) Monitoring

- IL-10 is not a direct mediator of CRS but still it acts as a regulatory cytokine that counterbalances pro-inflammatory responses such as IL-6 and TNF-α.
- During CRS, elevated levels of IL-10 may indicate an endogenous attempt to suppress inflammation and are thus monitored and observed alongside other cytokines.

4.2 Immune Modulation Biomarker

- IL-10 quantification is important for observation of the immunosuppressive environment in CGT trials, particularly in therapies that are involved in targeting autoimmune conditions or involving regulatory T cells.
- Helps in assessing immune tolerance and risk of immune-related adverse effects.

4.3 Genetic Modulation

- Gene-editing strategies incorporate IL-10 gene expression into cell-based therapies (e.g., Tregs, MSCs) to improve their anti-inflammatory potential.
- Engineered immune cells overexpressing IL-10 are being explored and extensively studies to treat graft-versus-host disease (GVHD), autoimmune disorders, and to promote immune tolerance in transplantation.

Scope of Validation

The PrecisionBind Human IL-10 (Catalog No KB1072) 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-10.

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-10 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 IL-10 ELISA kit is intended to measure the IL-10 (Interleukin-10) 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-10 Cmax Values and Recommended ELISA Range

This table summarizes IL-10 Cmax levels across diseases and suggests corresponding ELISA working ranges.

Application	Expected IL-10 Range (pg/ml)	Recommended ELISA Range (pg/ml)
Healthy Baseline	<5	0 - 50
Chronic Inflammatory Disease (RA, IBD, Psoriasis)	10 - 100 (peaks up to 300)	0 - 500

Sepsis / Cytokine Storm	50 - 31000 (can exceed 2000 in several cases)	0 - 2,000 (extendable to 5000)
Cell & Gene Therapy Cytokine Release Monitoring	20-800 (context dependent)	0–1000

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

The PrecisionBind Human IL-10 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 8 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-10 and are monoclonal antibodies. They show a high affinity to bind to native as well as recombinant IL-10

2.2 Selectivity
The ELISA has no or low cross reactivity to rat and mouse IL-10, IL-22 and IL-24.

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 15 U of 93/722 NIBSC-standard.
Therefore 1000 pg/ml is equivalent to 15 U of IL-10 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-10 ELISA = 2.97 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-10 ELISA - 9.01 pg/ml

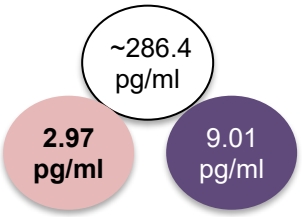
IC50 in ELISA (Half Maximal Inhibitory Concentration)
IC50 = The concentration of an inhibitor (drug, antibody, compound) required to reduce the signal (e.g., binding, enzymatic activity) by 50% compared to the maximum signal in the assay.

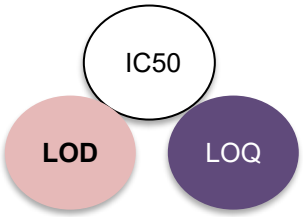
In ELISA, this is commonly used for:
Neutralization ELISA: Quantifies potency of antibodies inhibiting target–ligand interaction.
Drug Potency Testing: Measures concentration at which drug inhibits 50% of target activity.

IC50 for PrecisionBind Human IL-10 ELISA = ~286.4 pg/ml

Summary:

Parameter	Value (pg/mL)
LOD	2.97 pg/ml
LOQ	9.01 pg/ml
IC50	~286.4 pg/ml

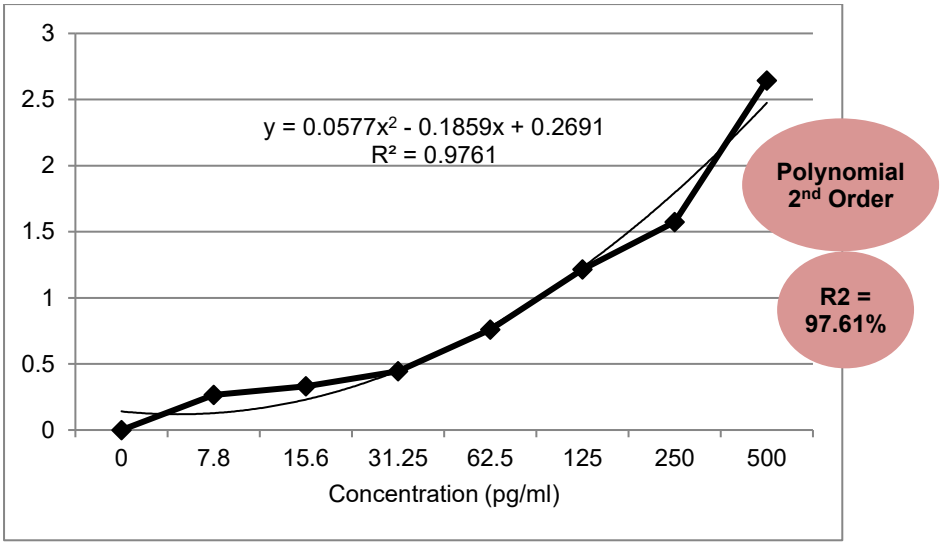


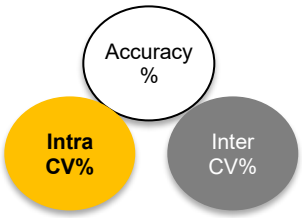


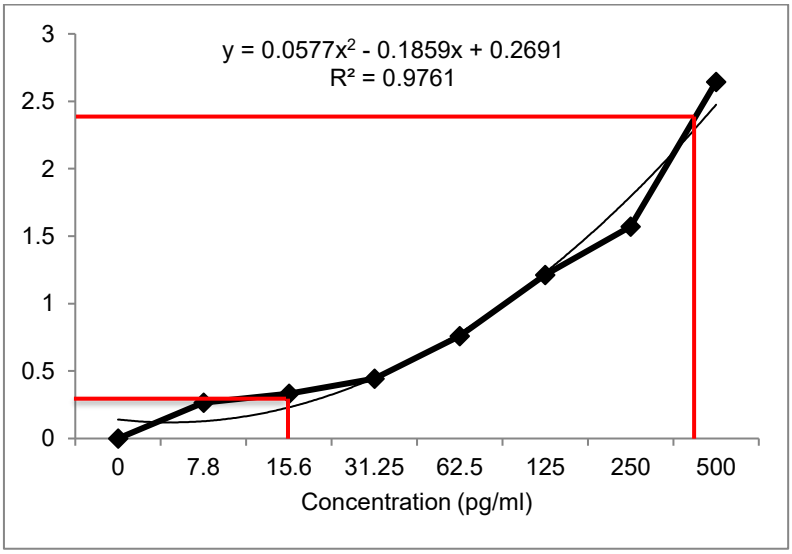
Regulatory Note:
LOD *S/N ≥ 3:1, LOQ ≥ 10:1, %CV ≤ 20% **S/N = Signal / Noise Ratio*

3. Linearity and Range

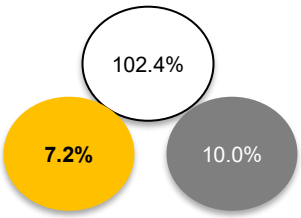
Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery
0	0.125	--	--
7.8	0.266	8	102.1
15.6	0.331	13.4	86.1
31.25	0.444	24.9	79.6
62.5	0.76	67.1	107.4
125	1.214	147.6	118.1
250	1.572	224.2	89.7
500	2.644	508	101.6
Positive QC (250 pg/ml)	1.609	221.3	88.5
Low QC (15.6 pg/ml)	0.378	16	102.4
High QC (400 pg/ml)	2.688	499.7	124.9







QC Low



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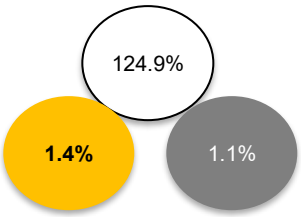


4. Accuracy and Precision (Intra / Inter-Assay)

A) Intra-Assay:

Standard Concentration (pg/ml)	Mean OD450	SD	%CV
7.8	0.237	1.40	7.2
125	1.254	2.44	2.4
500	2.597	2.88	1.4

B) Inter Assay:

Standard Concentration (pg/ml)	Mean OD450	SD	%CV
15.6	0.254	2.07	10.0
250	1.257	1.45	1.4
1000	2.588	2.35	1.1

5. Parallelism and Matrix Effect

Sample Dilution factor – Human Serum, Human Plasma and Human CSF samples have been tested. Neat samples can be run directly.

Neat Human Serum, Human Plasma and Human CSF were spiked with 500 pg/ml Human IL-10 and ELISA assay was run.

Sample	Mean OD450	Interpolated Concentration	% Recovery
Neat CSF samples	2.928	571.0	114.2
Neat Plasma	2.821	538.8	107.8
Neat Human Serum	2.863	551.4	110.3

A) Serum:

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:1	500	2.901	586.5	117.3	85.2
1:2	250	1.964	319.2	127.7	78.3
1:4	125	1.412	188.7	150.9	66.2
1:8	62.5	0.962	100.4	160.6	62.3
1:16	31.25	0.665	53.0	169.7	58.9
1:32	15.6	0.468	27.6	176.9	56.6
1:64	7.8	0.323	12.7	162.9	61.5

B) Plasma:

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:1	500	2.699	502.9	100.6	99.4
1:2	250	1.916	292.8	117.1	85.4
1:4	125	1.355	167.3	133.8	74.7
1:8	62.5	0.888	82.2	131.5	76.0
1:16	31.25	0.637	45.4	145.4	68.8
1:32	15.6	0.403	18.4	117.8	85.1
1:64	7.8	0.300	9.3	118.9	84.2

C) Cerebrospinal Fluid (CSF):

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:1	500	2.799	532.3	106.5	93.9
1:2	250	2.035	302.3	120.9	82.7
1:4	125	1.400	176.5	141.2	70.8
1:8	62.5	0.901	84.3	134.8	74.2
1:16	31.25	0.656	47.9	153.4	65.2
1:32	15.6	0.434	21.5	137.7	72.7
1:64	7.8	0.323	11.1	142.7	70.2

Results:

i) Parallelism is maintained across the 1:1 to 1:8 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 IL-10 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.

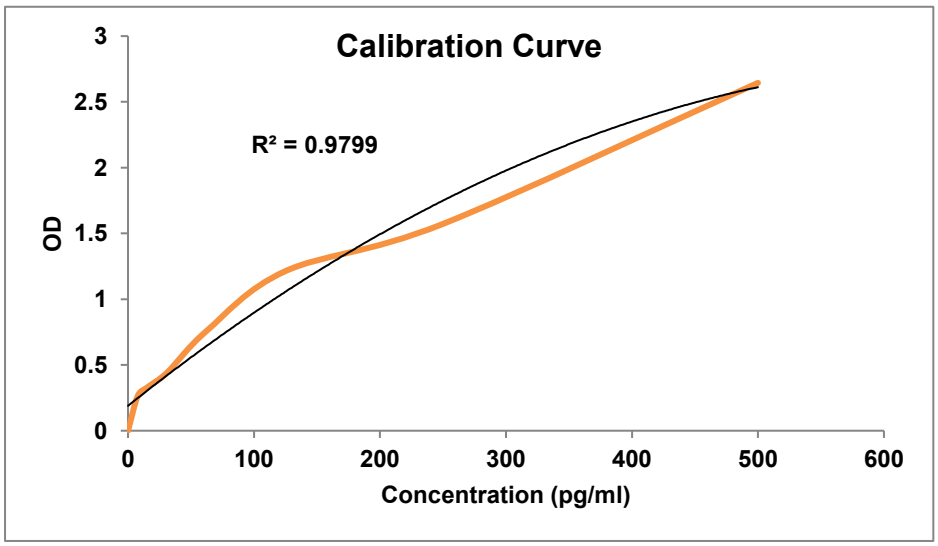
Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

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 93/722 IL-10

PrecisionBind Human IL-10 Kit Standard

Matrix Effect Heat Map

	1:1	1:2	1:4	1:8	1:16	1:32	1:64
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