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-15 ELISA KIT (Catalog No KBH0097) 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

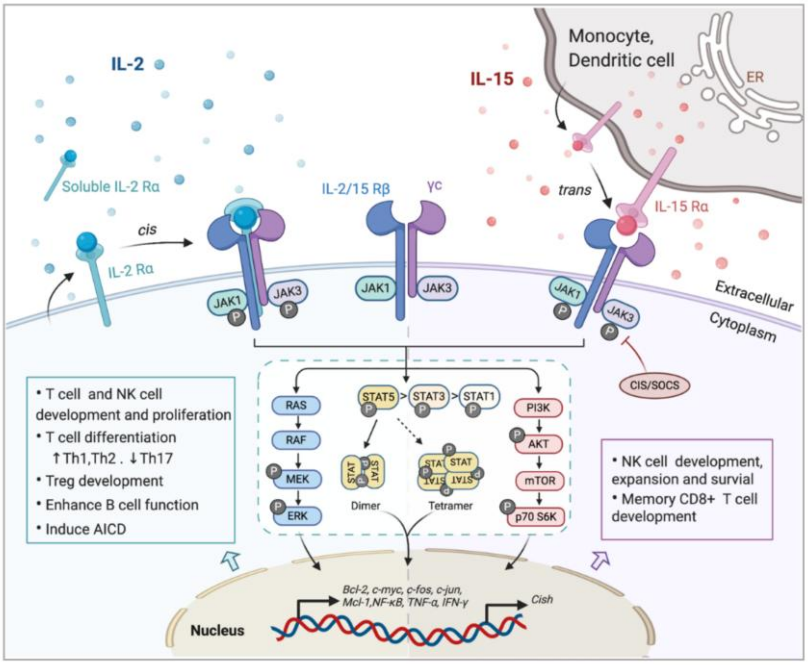
First Codification	History	Date
Version#1	**VALIDATION DATA OF PRECISIONBIND HUMAN IL-15 ELISA KIT (Catalog No KBH0097)**	31.07.2025

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

Background

1. Introduction to IL-15

Interleukin-15 (IL-15) is a type of pleiotropic pro-inflammatory cytokine secreted by dendritic cells, mononuclear phagocytes, and non-hematopoietic cells like epithelial and fibroblast-like cells. It plays a significant role in the development, survival, and activation of natural killer (NK) cells, $CD8^+$ memory T cells, and γδ T cells. IL-15 cascades through a heterotrimeric receptor complex comprising IL-15Rα, IL-2/15Rβ (CD122), and common γ chain (γc or CD132). Unlike many cytokines, IL-15 often operates via trans-presentation, where IL-15 bound to IL-15Rα on presenting cells that stimulates neighbouring cells expressing CD122/γc. Due to its potent role in immune-activating properties, IL-15 has emerged as a promising immunotherapeutic target in infectious diseases, cancer, and immunodeficiency syndromes.



2. Significance in Drug Discovery Research

2.1 Target Identification and Validation

- IL-15 is a primary cytokine that is involved in immune surveillance, especially in the maintenance and activation of NK cells and $CD8^+$ T cells.
- Dysregulation of IL-15 pathway leads to various pathological conditions including autoimmune diseases (e.g., celiac disease, rheumatoid arthritis), leukaemia, and transplant rejection.
- IL-15 is a validated and accepted target in cancer immunotherapy and antiviral strategies due to its affectivity in boosting cytotoxic lymphocyte activity.

2.2 Assay Development

- IL-15 is vastly used in the development of assays for immunomodulatory screening of drug:
  - ELISA and ECL assays for quantification of IL-15 levels.
  - Cell proliferation assays using CTLL-2 or NK-92 cells as functional readouts for IL-15 bioactivity.
  - Reporter gene assays to assess downstream signalling (e.g., STAT5 phosphorylation).

2.3 Biomarker for Efficacy and Safety

- The levels of IL-15 are associated with immune activation status and are thoroughly observed during immunotherapy.
- It potentially serves as a biomarker in inflammation-driven diseases and immune therapies so that it can balance efficacy with risk of cytokine-mediated toxicity.

3. Relevance in Biopharmaceutical Development

3.1 Monoclonal Antibodies and Biosimilars

- Recombinant IL-15 and IL-15 superagonists (e.g., N-803/ALT-803) are investigated in advanced clinical trials for solid tumours and haematological malignancies.
- Development involves:
  - IL-15:IL-15Rα binding affinity assays.
  - Potency assays based on proliferation of lymphocyte and cytokine secretion.
  - Comparability and stability studies for regulatory clearance and GMP production.

3.2 PK/PD Studies

- The IL-15 serum levels and subset analysis of immune cell (NK/$CD8^+$ T cell counts) work as pharmacodynamic (PD) markers.
- Pharmacokinetic (PK) studies have showcased IL-15 bioavailability, receptor occupancy, and in vivo half-life of engineered constructs.

3.3 Safety Evaluation

- Administration of high-dose IL-15 might induce cytokine-related toxicities including fever, hypotension, and CRS-like symptoms.
- Optimisation of effective dosage and cytokine profiling are significant in order to minimize severe effects during early-phase trials.

4. Importance in Cell and Gene Therapy (CGT)

4.1 Cytokine Release Syndrome (CRS) Monitoring

- IL-15 emphasises on CAR-T and CAR-NK cell persistence, survival, and cytolytic activity.

- Armored CAR constructs co-expressing IL-15 show superior anti-tumor responses in preclinical and clinical studies.
- Although less dominant than IL-6, IL-15 is involved in early-stage immune cell activation in CRS.
- Observation of IL-15 alongside other cytokines (e.g., TNF-α, IFN-γ) informs risk stratification and patient management.

4.2 Immune Modulation Biomarker

- Quantification of IL-15 is highly essential for strategizing immune responses during CGT clinical trials, particularly to assess the efficacy, persistence, and activation status of engineered immune cells such as CAR-T or CAR-NK cells.

4.3 Genetic Modulation

- Gene-editing strategies involves overexpression of IL-15 to enhance the proliferation, survival, and antitumor activity of adoptively transferred immune cells, or alternatively, modulate IL-15 signalling to prevent excessive activation of the immune system or autoimmunity in cell-based therapies.

Scope of Validation

The PrecisionBind Human IL-15/ IL15 ELISA (Catalog No KBH0097) 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-15.

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-15 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 IL-15 ELISA kit is intended to measure the IL-15 (Interleukin-15) 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-15 Cmax Values and Recommended ELISA Range

This table summarizes IL-15 Cmax levels across diseases and suggests corresponding ELISA working ranges.

Application	Expected IL-15 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 - 500 (spikes up to 1000)	0 - 1,000
Cell & Gene Therapy Cytokine Release Monitoring	100-1500 (can exceed 2000 during CRS)	0–3,000 (extendable to 5000)

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

The PrecisionBind Human IL-15 ELISA kit is developed using an assay range of 15.6 - 1000 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 64 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-15 and are monoclonal antibodies. They show a high affinity to bind to native as well as recombinant IL-15.

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 18 U of 95/554 NIBSC-standard.
Therefore 1000 pg/ml is equivalent to 18 U of IL-15 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-15 = 3.62 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-15 ELISA – 10.97 pg/ml

IC50 in ELISA (Half Maximal Inhibitory Concentration)

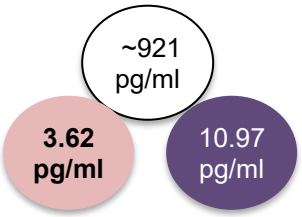
IC50 = The concentration of an inhibitor (drug, antibody, compound) required to reduce the signal (e.g., binding, enzymatic activity) by 50% compared to the maximum signal in the assay.

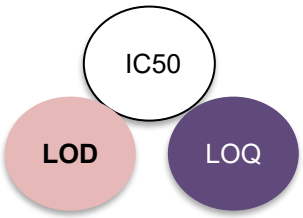
In ELISA, this is commonly used for:
Neutralization ELISA: Quantifies potency of antibodies inhibiting target–ligand interaction.
Drug Potency Testing: Measures concentration at which drug inhibits 50% of target activity.

IC50 for PrecisionBind Human IL-15 ELISA = ~921 pg/ml

Summary:

Parameter	Value (pg/mL)
LOD	3.62 pg/ml
LOQ	10.97 pg/ml
IC50	~ 921 pg/ml

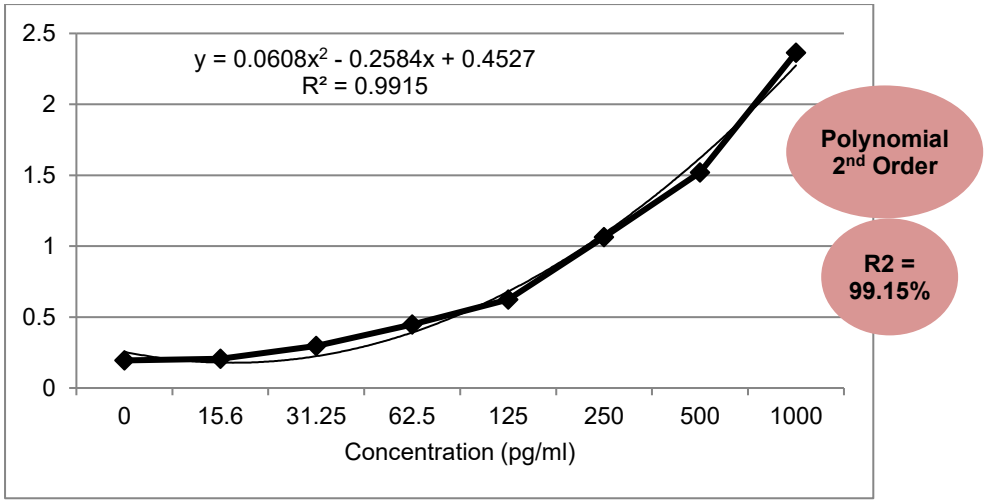


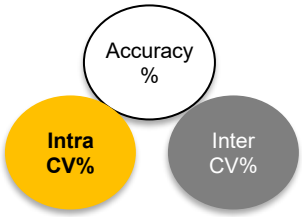


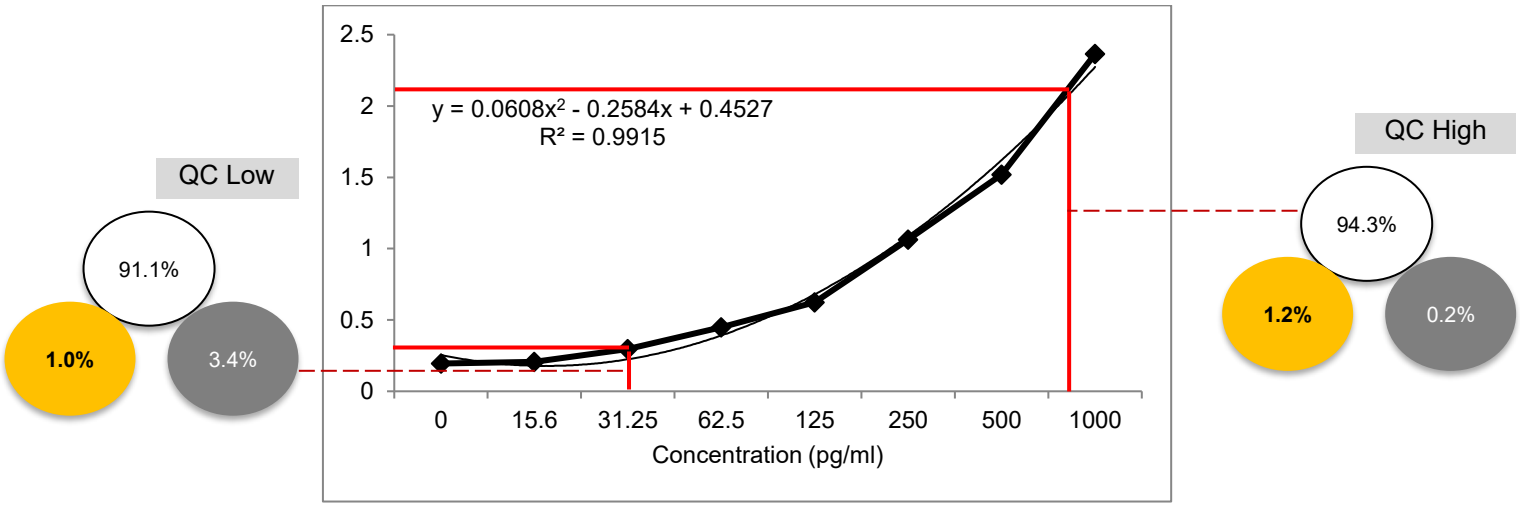
Regulatory Note:
LOD *S/N ≥ 3:1, LOQ ≥ 10:1, %CV ≤ 20% *S/N = Signal / Noise Ratio

3. Linearity and Range

Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery
0	0.195	0.4	--
15.6	0.207	15.9	102.2
31.25	0.296	26.9	86.0
62.5	0.449	49.8	79.6
125	0.623	134.2	107.4
250	1.063	295.3	118.1
500	1.519	448.4	89.7
1000	2.365	1016.0	101.6
Positive QC (750 pg/ml)	1.908	692.6	92.3
Low QC (31.25 pg/ml)	0.300	28.5	91.1
High QC (800 pg/ml)	2.007	754.6	94.3







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

A) Intra-Assay:

Standard Concentration (pg/ml)	Mean OD450	SD	%CV
15.6	0.199	0.16	1.0
250	0.682	2.84	5.1
1000	2.312	2.33	1.2

B) Inter Assay:

Standard Concentration (pg/ml)	Mean OD450	SD	%CV
15.6	0.205	0.70	3.4
250	0.717	4.57	6.4
1000	2.318	0.57	0.2

4. 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-15 and ELISA assay was run.

Sample	Mean Absorbance	Interpolated Concentration	% Recovery
Neat CSF samples	1.599	518.4	103.7
Neat Plasma	1.606	522.0	104.4
Neat Human Serum	1.615	526.7	105.3

A) Serum:

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:1	1000	2.321	974.0	97.4	102.7
1:2	500	1.597	517.3	103.5	96.7
1:4	250	1.066	275.0	110.0	90.9
1:8	125	0.634	121.7	97.4	102.7
1:16	62.5	0.480	75.8	121.2	82.5
1:32	31.3	0.333	36.5	116.8	85.5
1:64	15.6	0.237	14.0	89.7	111.7

B) Plasma:

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:1	1000	2.395	1031.4	103.1	97.0
1:2	500	1.539	487.6	97.5	102.5
1:4	250	1.058	271.9	108.7	92.0
1:8	125	0.673	134.1	107.3	93.2
1:16	62.5	0.500	81.5	130.4	76.7
1:32	31.3	0.325	34.6	110.4	90.4
1:64	15.6	0.244	15.5	99.5	100.6

C) Cerebrospinal Fluid (CSF):

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:1	1000	2.410	1043.3	104.3	95.8
1:2	500	1.633	536.2	107.2	93.2
1:4	250	1.043	265.9	106.4	94.0
1:8	125	0.663	130.9	104.7	95.5
1:16	62.5	0.488	78.0	124.9	80.1
1:32	31.3	0.330	35.8	114.4	87.3
1:64	15.6	0.245	15.7	101.0	99.2

Results:

i) Parallelism is maintained across the 1:1 to 1:64 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-15 ELISA kit was tested for matrix effect on human serum, plasma, CSF and physiological buffer 7.4 to mimic tear fluid samples.

5. 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.

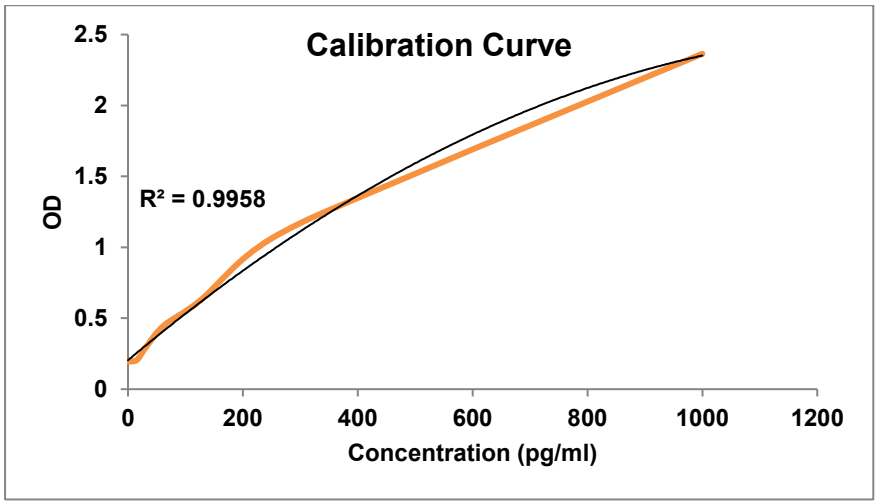
Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

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 95/554 IL-15

PrecisionBind Human IL-15 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