ELISA VALIDATION GUIDE

ASSAY FOR USE IN

DRUG DISCOVERY RESEARCH,
BIOPHARMA APPLICATIONS

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

VALIDATION OF GENLISA® MOUSE INTERLEUKIN-6 (IL-6/ IL6) ELISA KIT (CATALOG NO. KB2068) 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 GENLISA® MOUSE INTERLEUKIN-6 (IL-6/ IL6) ELISA (Cat No # KB2068)**	15.07.2026

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

Introduction

This document presents a discussion of the characteristics of our **GENLISA® Mouse Interleukin-6 (IL-6/ IL6) ELISA (Catalog No KB2068)** kit 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 **Mouse IL-6.**

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

- **Assay Validation**
- **Standard Curve**
- **Pharmacokinetic Relevance**
- **Precision and Reproducibility**
- **Linearity**

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

Background

Interleukin-6 (IL-6) is a pleiotropic pro-inflammatory cytokine produced by a variety of cells including macrophages, monocytes, T lymphocytes, B lymphocytes, fibroblasts, endothelial cells, and other immune and non-immune cells in response to infection, tissue injury, and immune stimulation. IL-6 plays a central role in the regulation of immune responses, inflammation, hematopoiesis, and acute-phase reactions. In mice, IL-6 is widely studied as a key mediator of inflammatory and autoimmune diseases, infectious disorders, cancer progression, and metabolic dysfunction. Elevated levels of Mouse IL-6 are associated with enhanced inflammatory activity and disease severity in numerous experimental models. Quantification of Mouse IL-6 is therefore important for investigating cytokine networks, monitoring inflammatory responses, evaluating disease progression, and assessing the efficacy of therapeutic interventions in preclinical research involving murine models.

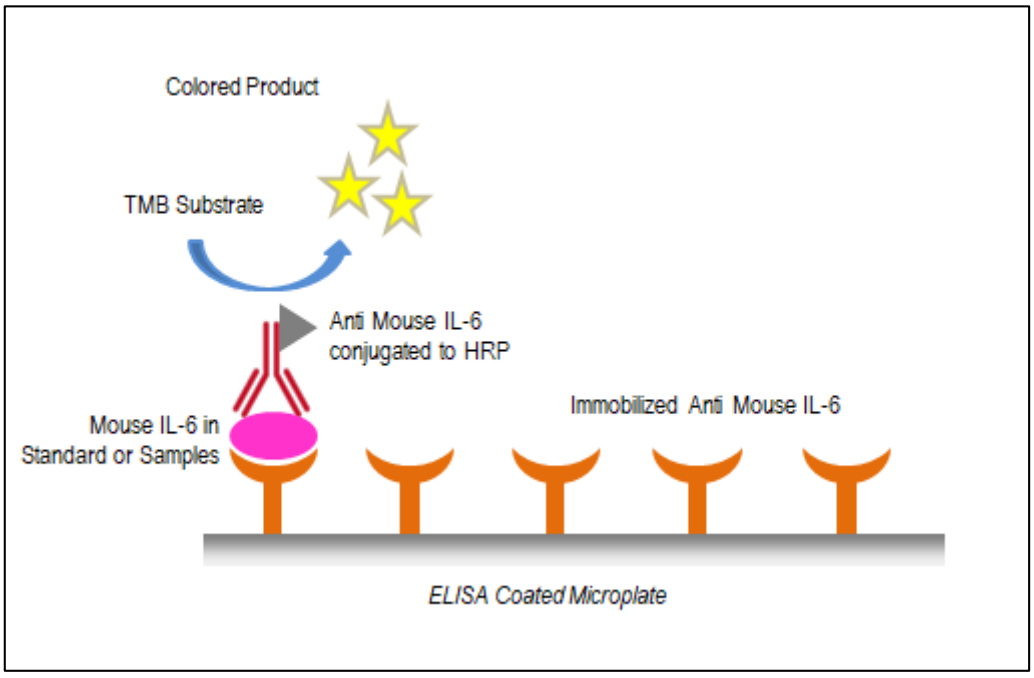
1. Purpose

To assess the specificity, assay performance, and clinical relevance of the GENLISA® Mouse Interleukin-6 (IL-6/IL6) ELISA developed using Anti Mouse IL-6 as capture protein.

2. Experimental Design

- `A sandwich ELISA was performed using anti-mouse IL-6 as capture protein.
- Standards prepared for Mouse IL-6.
- Assay Concentration Range: 0 - 2000 pg/ml.
- Signal (% absorbance) plotted versus concentration.

The GENLISA® Mouse Interleukin-6 (IL-6) ELISA employs a highly specific sandwich immunoassay strategy to ensure selective capture and detection of Mouse IL-6 present in biological samples. Affinity-purified anti-Mouse IL-6 antibodies immobilized on the microplate surface specifically bind Mouse IL-6 while preserving its native antigenic structure and epitope integrity. Following capture, an HRP-conjugated anti-Mouse IL-6 detection antibody binds to a distinct epitope on the cytokine, resulting in the formation of a stable antibody–antigen–antibody sandwich complex. Upon addition of the TMB substrate, the bound HRP enzyme catalyzes the development of a colored product, the intensity of which is directly proportional to the concentration of Mouse IL-6 present in the sample. Other cytokines and unrelated proteins exhibit minimal or no binding under these assay conditions, reflecting the high specificity of the antibody pair and ensuring reliable quantification of Mouse IL-6 in biological samples.



ELISA kits for Mouse IL-6 estimation offered by KRISHGEN uses Anti Mouse IL-6 capture proteins as above

3. Assay Validation

- IC50 Value : ~ 294.3 pg/ml (within 0-2000 pg/ml assay range).
- LLOQ : ~ 31.25 pg/ml.
- Clinical Cmax Values* : NA
- Mouse IL-6 is an endogenous cytokine produced by immune and non-immune cells and is not administered therapeutically; therefore, no fixed dose-dependent Cmax values are defined.
- Published studies indicate that Mouse IL-6 concentrations are generally low or undetectable under normal physiological conditions but can increase from low pg/mL levels

to several hundred or thousand pg/mL in response to infection, inflammation, autoimmune disorders, tissue injury, or other immune stimuli.

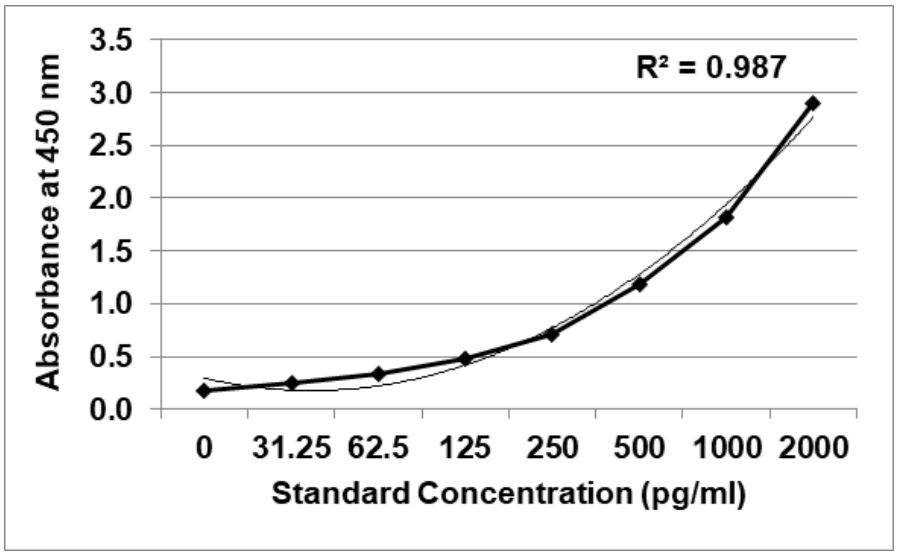
- Precision:
  - Intra-Assay CV: <10%.
  - Inter-Assay CV: <12%.
  - Inter-Operator CV: <10%.

4. Standard Curve

Below is the standard curve for Mouse IL-6 Sandwich ELISA assay:

Linearity and Range

Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration	%Interpolated Concentration against Actual Concentration
0	0.182	--	--
31.25	0.251	32.8	104.9
62.5	0.335	62.3	99.6
125	0.485	124.5	99.6
250	0.716	238.4	95.4
500	1.185	520.6	104.1
1000	1.815	987.6	98.8
2000	2.901	2002.7	100.1



5. LOD and LOQ

- LOD Absorbance : ~31 pg/ml
- LOQ Absorbance : ~31.25 pg/ml

6. Pharmacokinetic Relevance

The assay is designed to detect physiologically and pathologically relevant levels of Mouse Interleukin-6 (IL-6) in biological samples, making it suitable for cytokine profiling, inflammation studies, and immunological research applications.

The Mouse IL-6 ELISA demonstrates high sensitivity, typically within the pg/mL range, ensuring accurate detection and quantification of IL-6 across biologically meaningful concentrations.

Published studies evaluating Mouse IL-6 expression indicate variable cytokine levels depending on the disease model, inflammatory stimulus, and sample type:

• IL-6 concentrations are influenced by the degree of immune activation, infection status, inflammatory response, and experimental treatment conditions.

• Baseline IL-6 levels in healthy mice are generally low or undetectable, whereas significantly elevated concentrations may be observed in inflammatory, autoimmune, infectious, and tumor-associated models.

• Increased IL-6 levels are often associated with enhanced inflammatory activity, acute-phase responses, and disease progression.

Thus:

• Mouse IL-6 concentrations in serum, plasma, cell culture supernatants, and tissue homogenates typically fall within the measurable range of the ELISA.

• The assay working range enables reliable differentiation between basal, moderate, and elevated cytokine expression levels.

• In samples exhibiting high IL-6 concentrations, appropriate sample dilution may be required to ensure measurements fall within the linear dynamic range of the assay.

• The assay is therefore suitable for cytokine profiling, assessment of inflammatory responses, therapeutic efficacy studies, and biomarker research in mouse models.

7. Precision and Reproducibility

Precision was assessed by analysing three standard concentrations (62.5 pg/ml, 250 pg/ml, and 2000 pg/ml). Each concentration was tested in triplicate across three independent assay runs. %CV (Coefficient of Variation) was calculated within runs (intra-assay precision) and across runs (inter-assay precision).

Acceptance Criteria:

- Intra-assay %CV should be ≤15% for samples.
- Inter-assay %CV should be ≤15% for samples.
- %CV at LLOQ (Lower Limit of Quantitation) allowed up to 20%.

Precision Results Summary:

Standard (pg/ml)	Intra-Assay %CV (Range)	Inter-Assay %CV
62.5	1.8% to 10%	<12%
250	0.9% to 5%	<8%
2000	0.7% to 5%	<8%

Observations:

- Intra-assay precision was consistently less than 10% across all levels tested.
- Inter-assay precision was consistently less than 12%.
- All precision values met the acceptance criteria for ELISA validation.

Conclusion:
The GENLISA® Mouse Interleukin-6 (IL-6/IL6) ELISA demonstrates excellent intra- and inter-assay precision. These results support the assay's reliability and reproducibility for routine use in pharmacokinetic and bioanalytical studies.

8. Linearity

The linearity study was performed to evaluate the ability of the GENLISA® Mouse Interleukin-6 (IL-6/IL6) ELISA ELISA to generate results that are directly proportional to the concentration of Mouse IL-6 across a range of sample dilutions.

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%

Observations:

- Mouse IL-6 -spiked serum samples demonstrated recoveries ranging from 82–112% across the tested dilution series.
- Mouse IL-6 -spiked EDTA plasma samples demonstrated recoveries ranging from 83–118% across the tested dilution series.
- Mouse IL-6 -spiked heparin plasma samples demonstrated recoveries ranging from 80–99% across the tested dilution series.
- All matrices exhibited acceptable dilution linearity with recoveries within the predefined acceptance criteria.

Conclusion:
The GENLISA® Mouse Interleukin-6 (IL-6/IL6) ELISA exhibited acceptable linearity in serum, EDTA plasma, and heparin plasma matrices. The observed recoveries were within acceptable limits, demonstrating that the assay accurately measures Mouse IL-6 over a range of sample dilutions and is suitable for quantitative analysis of diluted biological samples.

9. Conclusion

The GENLISA® Mouse Interleukin-6 (IL-6/IL6) is validated for sensitivity, specificity, precision, and accuracy, and is appropriate for pharmacokinetic applications in regulatory settings.

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Version 1.0, dated 15.07.2026