# ELISA VALIDATION GUIDE

ASSAY FOR USE IN

DRUG DISCOVERY RESEARCH, METABOLIC AND

DIABETES APPLICATIONS

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

**VALIDATION OF GENLISA HUMAN GLP-1 ELISA KIT (CATALOG NO. KBH11042) 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 HUMAN GLP1 ELISA KIT (CATALOG NO. KBH11042)** | 31.12.2025 |
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| Approved Quality Control | Approved Product Development | Approved Operations Head |
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## Background

### 1. Introduction to Human Glucagon-Like Peptide-1 (GLP-1)

Glucagon-like peptide-1 (GLP-1) is an endogenous incretin hormone that plays a critical role in the regulation of glucose homeostasis and energy balance. It is derived from the post-translational processing of the proglucagon gene and is primarily secreted by enteroendocrine L-cells of the distal small intestine and colon in response to nutrient ingestion. Circulating GLP-1 acts through the GLP-1 receptor, a G-protein–coupled receptor expressed predominantly on pancreatic β-cells as well as in the gastrointestinal tract, central nervous system, heart, and other peripheral tissues.

Physiologically, GLP-1 enhances glucose-dependent insulin secretion, suppresses inappropriate glucagon release from pancreatic α-cells, slows gastric emptying, and promotes satiety. These coordinated actions contribute to postprandial glucose control while minimizing the risk of hypoglycaemia. In addition to its metabolic effects, GLP-1 has been implicated in β-cell preservation, cardiovascular protection, and modulation of appetite-regulating neural pathways, underscoring its broad role in metabolic regulation.

Native human GLP-1 exists mainly as GLP-1(7–36) amide and GLP-1(7–37), both of which are biologically active but are rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4), resulting in a short plasma half-life of approximately 1–2 minutes. This inherent instability limits the direct therapeutic use of endogenous GLP-1 and has driven the development of GLP-1 receptor agonists and DPP-4 inhibitors to enhance or prolong incretin activity in clinical practice.

From a mechanistic perspective, GLP-1 serves as a key model peptide for studying incretin biology, receptor signalling, and glucose-dependent endocrine regulation. Its structure–function relationships have been extensively characterized, providing insights into receptor binding domains, signal transduction pathways, and peptide degradation mechanisms. These foundational studies have informed the rational design of long-acting GLP-1 analogs with improved stability, receptor selectivity, and clinical efficacy.

In clinical, translational, and regulatory contexts, GLP-1 is central to the evaluation of incretin-based therapies for type 2 diabetes mellitus and obesity. Measurement of endogenous GLP-1 levels, assessment of receptor activation, and comparison of pharmacological agents against native GLP-1 activity are integral to drug development, biosimilars evaluation, and metabolic research. As the global burden of metabolic disorders continues to rise, GLP-1 remains a pivotal physiological hormone and a cornerstone target for next-generation therapeutic strategies.

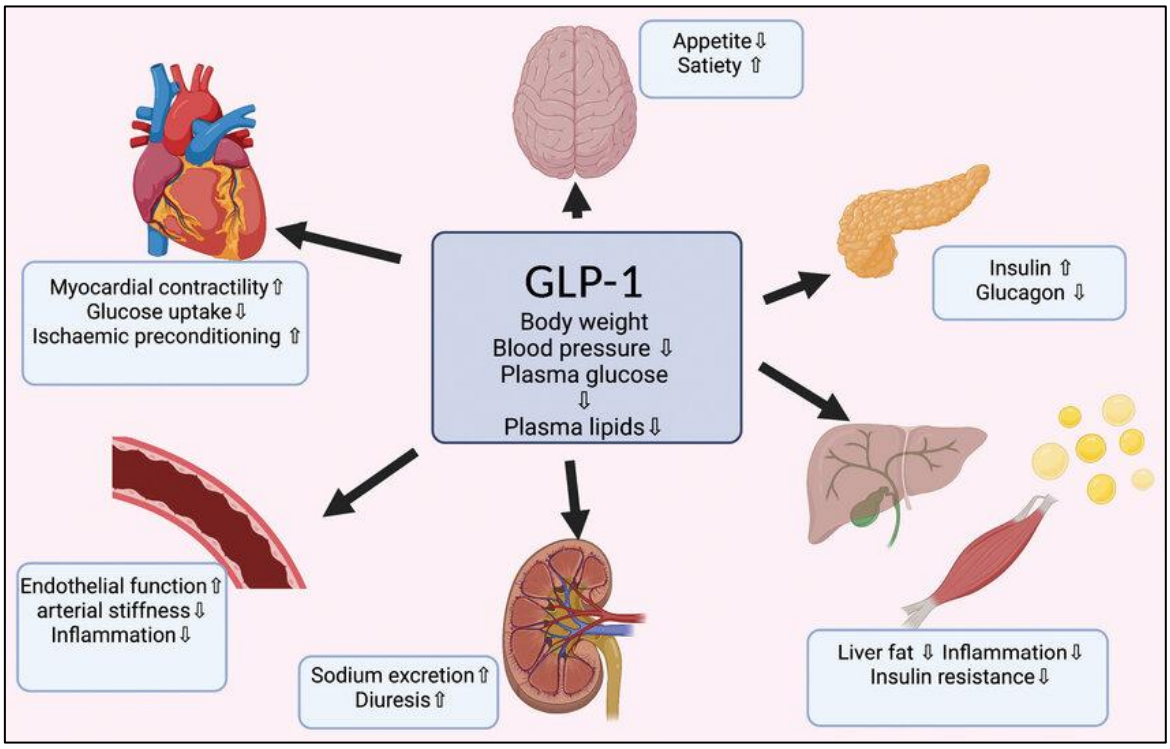



**Figure 1: Clinical response of GLP-1.**

## 2. Clinical Relevance of GLP-1 Based Monitoring

Monitoring glucagon-like peptide-1 (GLP-1) activity and related biomarkers is an important component of metabolic assessment in patients receiving incretin-based therapies, particularly in those demonstrating variable glycemic responses, suboptimal postprandial glucose control, or inconsistent therapeutic outcomes. As a central regulator of glucose-dependent insulin secretion, glucagon suppression, gastric emptying, and appetite, GLP-1 dynamics directly influence both short-term glycemic excursions and long-term metabolic control. Quantitative and functional assessment of GLP-1 therefore provides valuable insight into treatment responsiveness and underlying physiological mechanisms.

Evaluation of endogenous GLP-1 levels, receptor activation, and downstream metabolic effects enables correlation of incretin activity with clinically relevant outcomes such as postprandial glucose excursions, insulin secretory capacity, body-weight changes, and appetite regulation. Altered GLP-1 secretion or rapid enzymatic degradation by dipeptidyl peptidase-4 (DPP-4) may contribute to inadequate incretin responses, leading to impaired glucose tolerance or reduced therapeutic efficacy. GLP-1–based monitoring helps distinguish incretin-related dysfunction from other contributors to poor glycaemic control, including insulin resistance, β-cell failure, or lifestyle-related factors.

From a therapeutic management perspective, GLP-1 monitoring supports informed clinical decision-making regarding the selection, dosing, and optimization of incretin-based interventions, including GLP-1 receptor agonists and DPP-4 inhibitors. Assessment of GLP-1 activity can guide treatment intensification, switching between therapeutic classes, or combination strategies with insulin or oral antidiabetic agents. In long-term management, tracking GLP-1–mediated effects aids in evaluating treatment durability, metabolic benefits beyond glycaemic control, and inter-patient variability in response.

Ultimately, GLP-1–based monitoring facilitates a personalized approach to diabetes and metabolic disease management by integrating hormonal, metabolic, and clinical data. This strategy enhances understanding of individual incretin physiology, supports regulatory and clinical research evaluations of incretin-based therapies, and contributes to optimized treatment outcomes by aligning therapeutic interventions with patient-specific metabolic profiles.

## Scope of Validation

This document presents a discussion of the characteristics of our GENLISA Human GLP-1 ELISA KIT (CATALOG NO. KBH11042) 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 Human GLP-1.
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).
- Sample Handling and Storage Conditions.
- References Human GLP-1 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 recommended results 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

To evaluate the specificity, assay performance, and clinical relevance of the GENLISA Human Glucagon-Like Peptide-1 GLP-1 ELISA Kit, designed specifically to detect and quantify human GLP-1 with high sensitivity and specificity. This assay enables accurate measurement of endogenous GLP-1 levels in biological matrices, supporting detailed assessment of incretin physiology and GLP-1 dynamics in both healthy and diseased states. It facilitates clinical research, pharmacodynamic evaluation, and metabolic studies by enabling precise quantification of GLP-1 concentrations and their temporal changes in response to nutrient intake or therapeutic intervention.

The assay supports informed clinical and research decision-making by enabling identification of altered GLP-1 secretion, impaired incretin responses, or variability in hormonal regulation that may contribute to suboptimal glycemic control. It is particularly valuable for evaluating the pharmacological effects of incretin-based therapies, including GLP-1 receptor agonists and DPP-4 inhibitors, as well as for comparative and translational studies. By providing reliable and reproducible GLP-1 measurements, the GENLISA Human GLP-1 ELISA Kit contributes to improved understanding of metabolic regulation, optimized treatment strategies for diabetes and obesity, and robust support for long-term clinical research and regulatory evaluation of incretin-based interventions.

## Principle of the Assay

This assay is based on a sandwich enzyme-linked immunosorbent assay (ELISA) principle. Microplate wells are pre-coated with Glucagon capture antibodies specific to the Human Glucagon-Like Peptide-1 (GLP-1). Human Glucagon Standards are added to the wells and incubated, allowing the Human Glucagon-Like Peptide-1 (GLP-1) present to bind specifically to the immobilized capture antibodies. After washing to remove unbound components, Human Glucagon biotin-labeled detection antibody specific to a different epitope of the Human Glucagon-Like Peptide-1 (GLP-1) is added. This detection antibody binds to the captured Human Glucagon-Like Peptide-1 (GLP-1), forming an antibody–antigen–antibody sandwich complex. Following another wash step, streptavidin conjugated to horseradish peroxidase (HRP) is added, which binds with high affinity to the biotin moiety on the detection antibody. Subsequent washing removes excess streptavidin-HRP, and tetramethylbenzidine (TMB) substrate is added. The HRP catalyzes a colorimetric reaction, resulting in the development of a blue color that turns yellow upon addition of stop solution. The optical density (OD) is measured at 450 nm, and the color intensity is directly proportional to the concentration of the Human Glucagon-Like Peptide-1 (GLP-1) present in the samples or standards.

## Experimental Design

- A Sandwich ELISA was performed using Glucagon capture antibodies as the capture antibody.
- Standards were prepared using purified Human Glucagon reference material.
- Assay Concentration Range: 0 - 2000 pg/ml.
- Signal (% absorbance) plotted versus concentration.
- The optimized antibody-coating and detection strategy employed in the GENLISA Human GLP-1 ELISA ensures efficient and specific capture of circulating GLP-1 while minimizing background signals from endogenous plasma proteins, structurally related peptides, or other serum components. This highly selective assay design enables sensitive and accurate quantification of GLP-1 in complex biological matrices, making it well suited for research applications as well as metabolic and endocrine studies involving glucose homeostasis and incretin hormone regulation.

The GENLISA Human GLP-1 ELISA utilizes a quantitative sandwich immunoassay format based on the selective interaction between Glucagon capture antibodies that recognize Human Glucagon-Like Peptide-1 with high affinity. Glucagon capture antibodies are pre-coated onto microwells to immobilize the target analyte. Standards and test samples are added, allowing GLP-1 present in the samples to bind to the coated capture antibodies. A biotin-labeled Human Glucagon, GLP-1-specific detection antibody is then applied, forming a stable antibody–antigen–antibody sandwich complex. Following a wash step to remove unbound components, streptavidin conjugated to horseradish peroxidase (HRP) is added, which binds to the biotinylated detection antibody. After additional washing, tetramethylbenzidine (TMB) substrate is added, producing a colorimetric signal proportional to the concentration of GLP-1 in the sample. The enzymatic reaction is stopped by the addition of stop solution, and absorbance is measured at 450 nm, enabling reliable quantitative determination of Human GLP-1 levels in biological samples.

### Validation Parameters and Acceptance Criteria

### 1. Human GLP-1 antibodies Values and Recommended ELISA Range

This table summarizes Human GLP-1 levels across different therapies and suggested corresponding ELISA working ranges.

| Application | Expected Human GLP-1 Range (pg/ml) | Recommended ELISA Range (pg/ml) |
|---|---|---|
| Baseline / fasting assessment (physiological reference) | 1-10 | 0–20 |

| Application | Expected Human GLP-1 Range (pg/ml) | Recommended ELISA Range (pg/ml) |
|---|---|---|
| Postprandial response evaluation (normal incretin secretion) | 10-40 | 0–50 |
| Enhanced GLP-1 activity (DPP-4 inhibitor therapy or metabolic stimulation) | 20-80 | 0–100 |
| Pharmacological elevation / GLP-1 receptor agonist studies or challenge tests | 50-300 | 0–500 |

Note: An assay sensitivity of ≤2 pg/mL is recommended for reliable detection of basal or fasting circulating Human GLP-1 levels, while an upper quantification limit in the range of ≥200–300 pg/mL is advised to accommodate postprandial elevations and pharmacologically stimulated GLP-1 responses. This analytical range supports accurate assessment of incretin secretion dynamics, metabolic status, and therapeutic modulation in physiological and clinical research settings.

The GENLISA Human Glucagon-Like Peptide-1 (GLP-1) ELISA kit is developed using an assay range of 0 - 2000 pg/ml with the dilutional linearity accuracy to measure responses as per the application table above on patient $C_{max}$ values. The kit has also been validated upto 32 fold dilution and the values are within the acceptable range.

## 2. Specificity and Selectivity

### 2.1 Specificity

The capture and detection reagents used in the Human GLP-1 ELISA are highly specific antibodies and assay components designed to selectively recognize human GLP-1 with minimal cross-reactivity to structurally related peptides or unrelated circulating hormones. The assay is optimized to detect GLP-1–specific epitopes, including sequence and conformational determinants characteristic of the biologically active GLP-1 peptide, ensuring high-affinity and selective binding of GLP-1 present in biological samples.

The specificity profile enables accurate discrimination of human GLP-1 in complex biological matrices such as serum or plasma while minimizing interference from endogenous plasma proteins, assay matrix components, or peptide degradation products. The assay demonstrates minimal cross-reactivity with other proglucagon-derived peptides or incretin-related hormones, including glucagon, GLP-2, gastric inhibitory polypeptide (GIP), oxyntomodulin, proglucagon fragments, or unrelated gastrointestinal and pancreatic hormones commonly present in metabolic samples.

This high degree of molecular specificity ensures reliable detection and quantification of human GLP-1 across a wide physiological and pharmacodynamic concentration range, including samples collected under fasting, postprandial, or pharmacologically stimulated

conditions. Consequently, the assay supports robust evaluation of incretin physiology, metabolic research, and clinical assessment of GLP-1–related pathways, while ensuring accurate and reproducible measurement in the presence of complex hormonal and metabolic backgrounds.

### 2.2 Selectivity

The Human GLP-1 ELISA demonstrates minimal to no cross-reactivity with structurally related incretin hormones, proglucagon-derived peptides, or unrelated circulating metabolic regulators. The assay selectively detects human GLP-1–specific antigenic determinants and effectively excludes peptides that do not share the unique amino acid sequence and conformational features of GLP-1, including regions critical for GLP-1 receptor interaction and biological activity.

The assay maintains high selectivity in complex biological matrices such as human serum, plasma, or cell-culture supernatants, with negligible interference from endogenous plasma proteins, peptide degradation products, or matrix-associated binding interactions. Minimal cross-reactivity is observed with glucagon, GLP-2, gastric inhibitory polypeptide (GIP), oxyntomodulin, proglucagon fragments, or other gastrointestinal and pancreatic hormones commonly present in metabolic or diabetic patient samples. Where applicable, the assay also demonstrates limited recognition of structurally modified GLP-1 receptor agonists, ensuring selective measurement of native human GLP-1.

This stringent selectivity profile ensures reliable and accurate quantification of human GLP-1 without false-positive signals arising from related peptides, therapeutic agents, or biologically active matrix components, thereby supporting robust assessment of incretin physiology, pharmacodynamic studies, and clinical research applications involving GLP-1–mediated metabolic regulation.

### 2.3 LOD, LOQ and $IC_{50}$

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 GENLISA Human Glucagon-Like Peptide-1 (GLP-1) ELISA = 16.71 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).

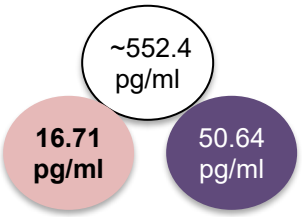
LOQ for GENLISA Human Glucagon-Like Peptide-1 (GLP-1) ELISA – 50.64 pg/ml

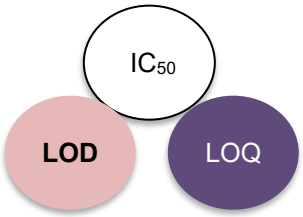
$IC_{50}$ in ELISA (Half Maximal Inhibitory Concentration)
$IC_{50}$ = The concentration of an inhibitor (drug, antibody, compound) required to reduce the signal (e.g., binding, enzymatic activity) by 50% compared to the maximum signal in the assay.

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.

$IC_{50}$ for GENLISA Human Glucagon-Like Peptide-1 (GLP-1) ELISA = ~552.4 pg/ml
Summary:

| Parameter | Value (pg/ml) |
|---|---|
| LOD | 16.71 pg/ml |
| LOQ | 50.64 pg/ml |
| $IC_{50}$ | 552.4 pg/ml |



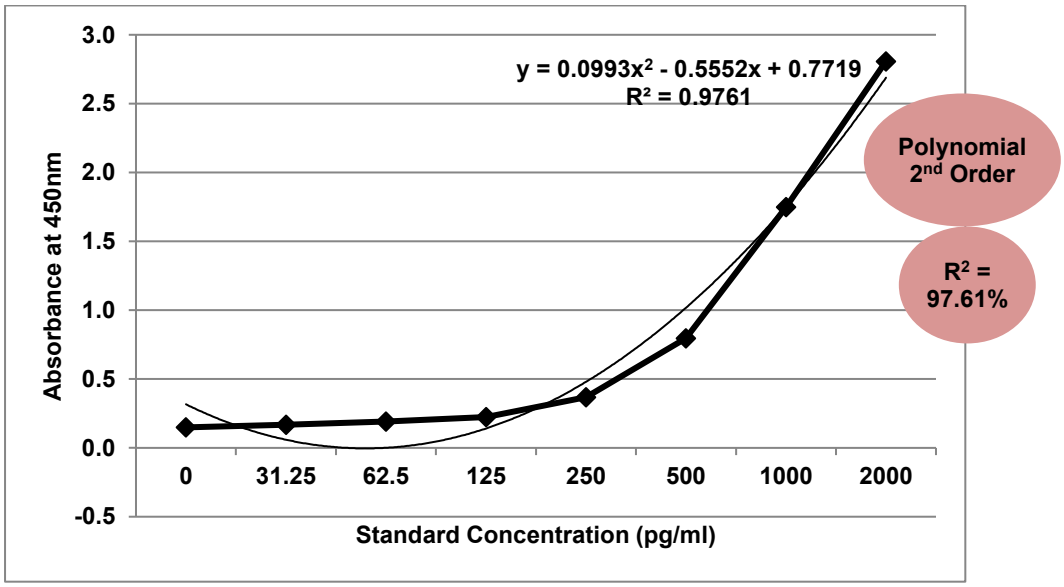



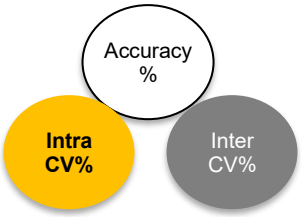

Regulatory Note:
LOD S/N ≥ 3:1, LOQ ≥ 10:1, %CV ≤ 20%  **S/N = Signal / Noise Ratio*

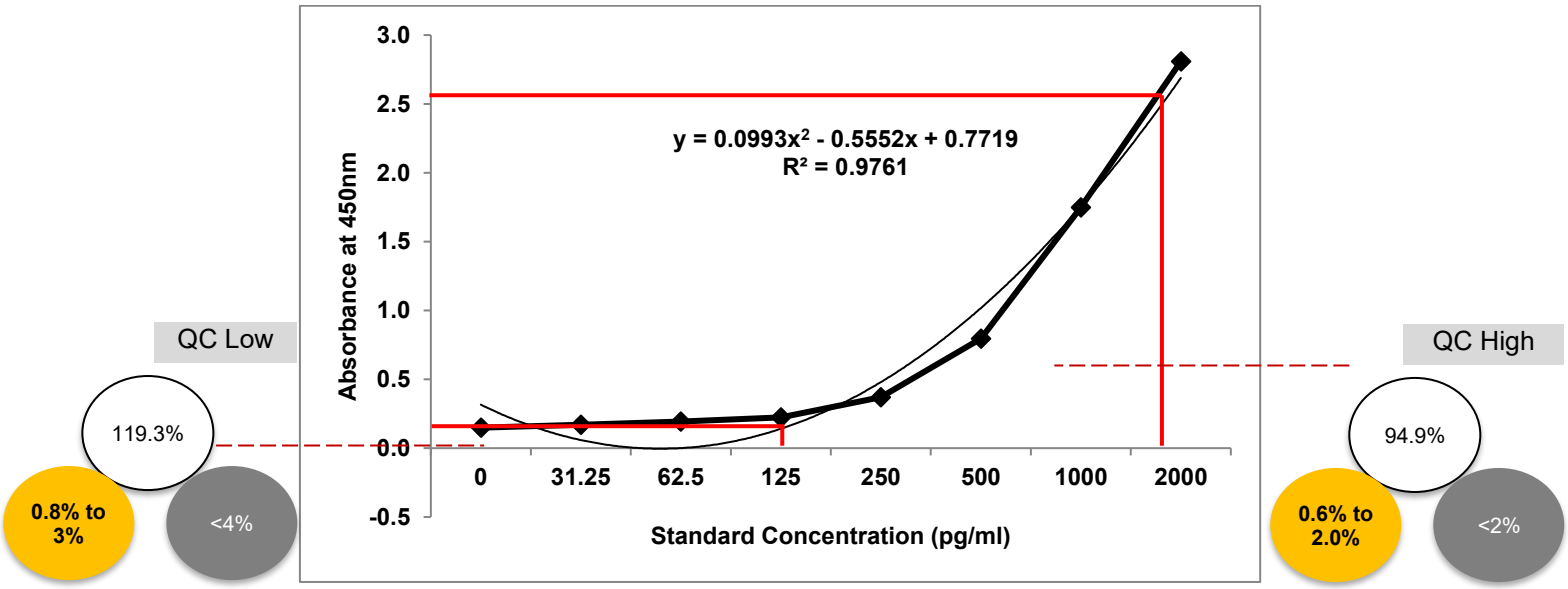
**3. Linearity and Range**

| Standard Concentration (pg/ml) | Mean Absorbance | Interpolated Concentration (pg/ml) | % Recovery |
|---|---|---|---|
| 0 | 0.149 | -- | -- |
| 31.25 | 0.169 | 17.2 | 54.9 |
| 62.5 | 0.193 | 68.1 | 109 |
| 125 | 0.223 | 126.3 | 101.1 |
| 250 | 0.369 | 255 | 102 |
| 500 | 0.797 | 496.8 | 99.4 |
| 1000 | 1.749 | 1001.6 | 100.2 |

| Standard Concentration (pg/ml) | Mean Absorbance | Interpolated Concentration (pg/ml) | % Recovery |
|---|---|---|---|
| 2000 | 2.808 | 1999 | 100 |
| Positive Control (1500 pg/ml) | 2.321 | 1689.4 | 112.6 |
| Low QC Control (125 pg/ml) | 0.243 | 149.1 | 119.3 |
| High QC Control (1800 pg/ml) | 2.599 | 1707.5 | 94.9 |




## 4. Precision and Reproducibility (Intra/Inter-Assay)

Precision was assessed by analyzing three standard concentrations (125 pg/ml, 500 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 QC samples.
- Inter-assay %CV should be ≤15% for QC 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 |
|---|---|---|
| 125 | 0.8% to 3% | <3% |
| 500 | 1.2% to 4% | <4% |
| 2000 | 0.6% to 1.7% | <2% |

Observations:

- Intra-assay precision was consistently less than 7% across all levels tested.
- Inter-assay precision was consistently less than 7%.
- All precision values met the acceptance criteria for ELISA validation.

Conclusion:
The GENLISA Human Glucagon-Like Peptide-1 (GLP-1) demonstrates excellent intra- and inter-assay precision. These results support the assay's reliability and reproducibility for routine use in pharmacokinetic and bio analytical studies.

**5. Diluents Effect Study**

Evaluation of PBS-based buffer vs Proprietary buffer revealed slight recovery differences. PBS (pH 7.4) diluent offered consistent and reliable performance across tested concentrations.

**6. Parallelism and Matrix Effect**

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

Neat Human Serum and Human Plasma were spiked with 2000 pg/ml Human GLP-1 and ELISA assay was run.

| Sample | Mean Absorbance | Interpolated Concentration | % Recovery |
|---|---|---|---|
| **Neat Human Plasma** | 1.757 | 1006.7 | 100.7 |
| **Neat Human Serum** | 1.789 | 1026.5 | 102.6 |

Serial dilutions of a high-concentration sample were prepared at dilutions of 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64 for both human serum and human plasma. Each dilution was assayed using the GENLISA Human Glucagon-Like Peptide-1 (GLP-1) and compared to the standard curve.

Acceptance Criteria:

- The back-calculated concentration (interpolated) should fall within ±20% of the expected concentration across the tested range.

- % Recovery should be between 80% and 120% for most samples.

**A) Human Serum:**

| Dilution | Expected Standard Concentration (pg/ml) | Mean Absorbance | Interpolated Concentration (pg/ml) | % Recovery | % Deviation |
|---|---|---|---|---|---|
| 1:2 | 1000 | 1.736 | 993.9 | 99.4 | 100.6 |

| Dilution | Expected Standard Concentration (pg/ml) | Mean Absorbance | Interpolated Concentration (pg/ml) | % Recovery | % Deviation |
|---|---|---|---|---|---|
| 1:4 | 500 | 0.745 | 470.2 | 94.0 | 106.3 |
| 1:8 | 250 | 0.378 | 259.3 | 103.7 | 96.4 |

| Dilution | Expected Standard Concentration (pg/ml) | Mean Absorbance | Interpolated Concentration (pg/ml) | % Recovery | % Deviation |
|---|---|---|---|---|---|
| 1:16 | 125 | 0.239 | 144.8 | 115.9 | 86.3 |
| 1:32 | 62.5 | 0.198 | 94.1 | 150.6 | 66.4 |
| 1:64 | 31.25 | 0.149 | --- | 0 | --- |

Results:

i. Parallelism is generally maintained across the 1:2 to 1:32 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 GENLISA Human Glucagon-Like Peptide-1 (GLP-1) ELISA kit was tested for matrix effect on human serum.

**B) Human Plasma:**

| Dilution | Expected Standard Concentration (pg/ml) | Mean Absorbance | Interpolated Concentration (pg/ml) | % Recovery | % Deviation |
|---|---|---|---|---|---|
| 01:02 | 1000 | 1.844 | 1061.3 | 106.1 | 94.2 |
| 01:04 | 500 | 0.737 | 466.1 | 93.2 | 107.3 |
| 01:08 | 250 | 0.384 | 263.4 | 105.4 | 94.9 |
| 01:16 | 125 | 0.341 | 132.9 | 106.3 | 94.1 |
| 01:32 | 62.5 | 0.183 | 68.5 | 109.6 | 91.2 |
| 1:64 | 31.25 | 0.157 | --- | --- | --- |

Results:

i. Parallelism is generally maintained across the 1:2 to 1:32 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 GENLISA Human Glucagon-Like Peptide-1 (GLP-1) ELISA kit was tested for matrix effect on human plasma.

Conclusion:

Parallelism was demonstrated between the diluted samples and the standard curve. This supports the validity of using sample dilutions within the working range of the Human Glucagon-Like Peptide-1 (GLP-1) ELISA without significant loss of accuracy.

## 7. 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.

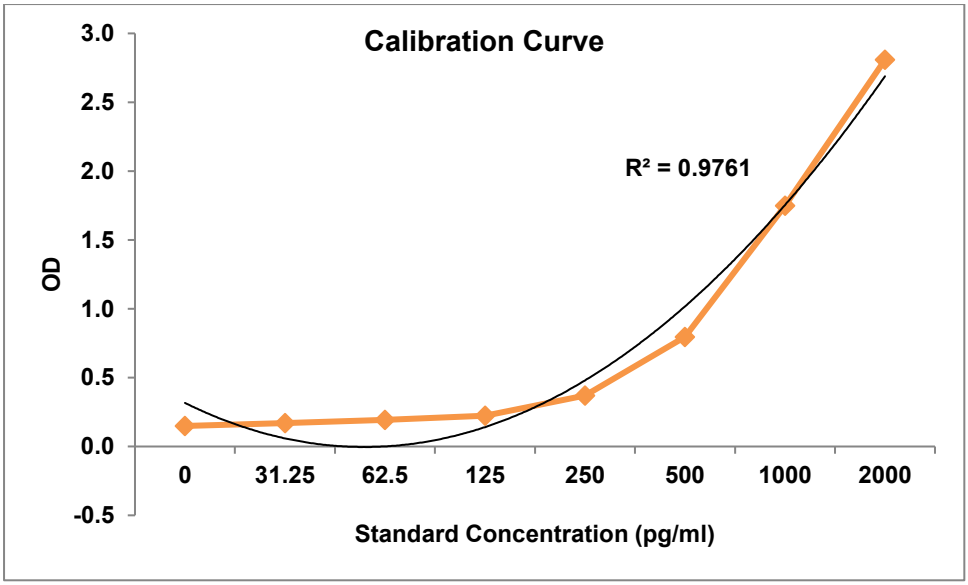
Store recombinant lyophilized standard at 2-8°C. Upon reconstitution aliquot standards into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.

Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

### C) Health Hazard Warnings:

- Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
- For Research Use Only.

### Graphs, Maps and Appendices:




### Matrix Effect Heat Map

| | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 |
|---|---|---|---|---|---|---|
| Serum | | | | | | |
| Plasma | | | | | | |

**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% | H (null hypothesis) = 100 ± 25 % |
| Parallelism/Linearity | CV < 30% | Deviation from linearity < 20% |
| LLOQ / LOQ | Recovery 100 ± 25% | TE % < 32.9% |

## References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