ELISA VALIDATION GUIDE

KRIBIOLISA® ANTI-LIRAGLUTIDE ELISA

Background

1. Introduction to Anti Liraglutide Antibodies

Anti-Liraglutide antibodies are immunoglobulins generated by the host immune system in response to exposure to Liraglutide, a long-acting glucagon-like peptide-1 (GLP-1) receptor agonist used in the management of type 2 diabetes mellitus and chronic weight control. As a therapeutic peptide with structural similarity to endogenous GLP-1 but modified for prolonged activity, Liraglutide can elicit an immune response in certain individuals, leading to the formation of binding and, in some cases, neutralizing anti-drug antibodies (ADAs).

These antibodies may recognize epitopes on the Liraglutide peptide backbone, its acylated side chain, or conformational structures formed upon albumin binding. The development of anti-Liraglutide antibodies has the potential to alter pharmacokinetics, reduce drug bioavailability, attenuate GLP-1 receptor activation, or interfere with downstream metabolic effects such as insulin secretion, glucagon suppression, gastric emptying delay, and appetite regulation. While most detected antibodies are non-neutralizing and transient, their presence is clinically relevant for long-term therapy monitoring and immunogenicity risk assessment.

From a mechanistic perspective, anti-Liraglutide antibodies serve as valuable tools for investigating immunogenicity, structure–immunoreactivity relationships, and therapeutic peptide stability. In clinical and research settings, their detection and characterization are performed using immunoassays such as bridging ELISA, competitive ELISA, electrochemiluminescence assays, and cell-based neutralization assays. These methodologies enable sensitive and specific measurement of ADA incidence, titer, and neutralizing capacity in treated populations.

Anti-Liraglutide antibodies are particularly important in regulatory and biosimilar development contexts, where immunogenicity profiling is a critical component of comparability and safety evaluation. Monitoring ADA responses supports pharmacovigilance, helps interpret inter-patient variability in therapeutic response, and informs dose optimization strategies. In preclinical and translational research, these antibodies contribute to the understanding of immune tolerance to peptide therapeutics and the mitigation of immune-mediated loss of efficacy.

With the expanding clinical use of GLP-1 receptor agonists and the development of next-generation incretin-based therapies, anti-Liraglutide antibodies remain a key analytical and research reagent for immunogenicity assessment, assay validation, and long-term safety evaluation in metabolic disease therapeutics.

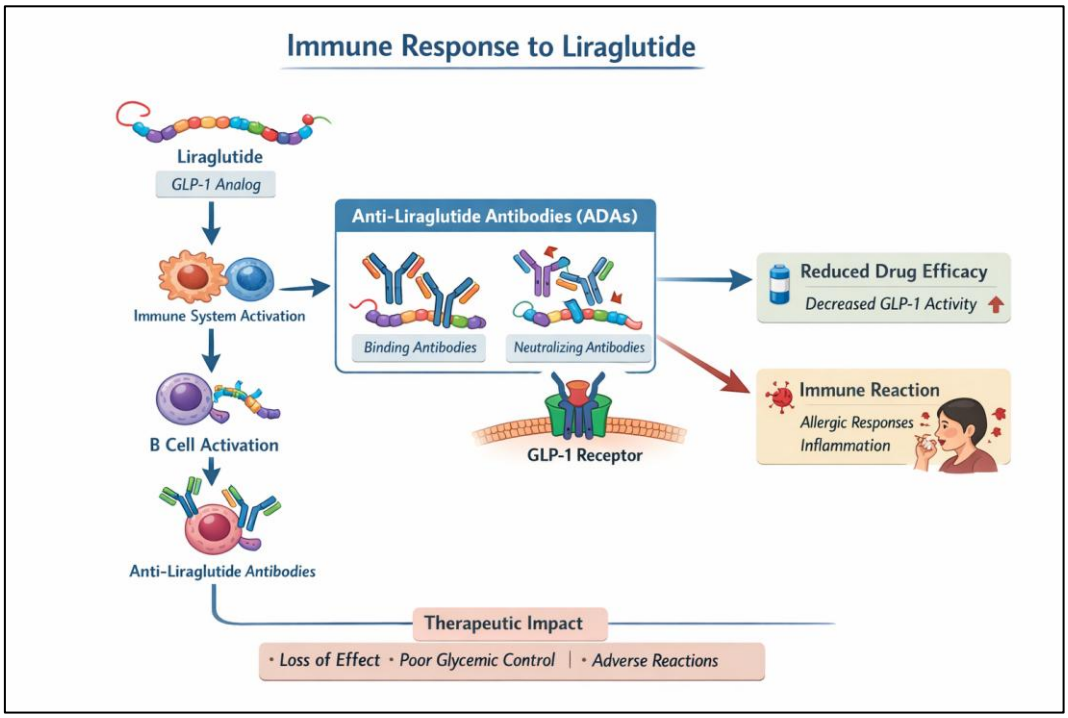


Figure 1: Immune response to Liraglutide.

2. Clinical Relevance of Anti Liraglutide-Based Monitoring

Monitoring anti-Liraglutide antibodies is a critical component of immunogenicity assessment during Liraglutide therapy, particularly in patients exhibiting variable glycaemic control, unexpected loss of therapeutic response, or altered pharmacokinetic behavior. The development of anti-drug antibodies (ADAs) can influence drug bioavailability, receptor engagement, and metabolic efficacy by accelerating clearance, reducing free circulating Liraglutide levels, or neutralizing GLP-1 receptor activation. Quantification of anti-Liraglutide antibodies therefore provides essential insight into treatment-related immune responses and their potential clinical impact.

Assessment of ADA incidence, titer, and persistence enables correlation of immune reactivity with clinically relevant outcomes such as diminished HbA1c reduction, suboptimal fasting and postprandial glucose control, attenuated weight loss, or increased dose requirements. In certain cases, neutralizing anti-Liraglutide antibodies may compromise incretin-mediated insulin secretion and glucagon suppression, leading to reduced therapeutic effectiveness despite adequate dosing. ADA monitoring also aids in distinguishing immunogenicity-driven treatment failure from disease progression or non-adherence.

From a therapeutic management perspective, anti-Liraglutide antibody monitoring supports informed clinical decision-making, including dose adjustment, treatment continuation, or transition to alternative GLP-1 receptor agonists with distinct molecular structures or immunogenicity profiles. In long-term treatment settings, immunogenicity data help identify patients at risk of sustained antibody responses that may affect drug durability or safety.

Ultimately, anti-Liraglutide–based monitoring enables a personalized treatment approach by integrating immunogenicity profiles with patient-specific metabolic responses and therapeutic outcomes. This strategy enhances the long-term effectiveness of Liraglutide therapy, supports regulatory pharmacovigilance requirements, and contributes to optimized clinical management of metabolic disorders while minimizing immune-mediated loss of efficacy.

Scope of Validation

This document presents a discussion of the characteristics of our KRIBIOLISA® Anti-Liraglutide ELISA KIT (CATALOG NO. KBI9040) 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 Anti-Liraglutide antibodies.
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).
- Accelerated Stability Study
- Sample Handling and Storage Conditions.
- References Anti-Liraglutide 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 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

To evaluate the specificity, assay performance, and clinical relevance of the KRIBIOLISA® Anti-Liraglutide ELISA Kit, designed specifically to detect and quantify anti-Liraglutide antibodies with high sensitivity and specificity. This assay enables accurate immunogenicity assessment during Liraglutide therapy by supporting the detection, characterization, and monitoring of anti-drug antibody (ADA) responses. It facilitates clinical research, pharmacovigilance, and treatment evaluation by enabling assessment of ADA incidence, titer, and persistence, as well as their potential impact on drug exposure and therapeutic efficacy. The assay strengthens clinical decision-making by supporting identification of immune-mediated loss of response, guiding dose optimization or therapeutic switching, and ensuring long-term treatment effectiveness in patients receiving Liraglutide for metabolic disorders such as type 2 diabetes mellitus and obesity.

Principle of the Assay

This ELISA is based on an Indirect immunoassay format. Liraglutide capture antibodies are immobilized onto 96-well microplate wells. Liraglutide Antibody, Rabbit Polyclonal, AF Purified present in the standards and test samples specifically binds to the coated antibodies during incubation. After a wash step to remove unbound substances, an Anti-Human IgG: HRP-conjugated detection antibody is introduced, which binds to the captured Liraglutide, forming a stable immune complex. Following additional washing to eliminate excess conjugate, TMB substrate is added, allowing HRP to catalyze a colorimetric reaction. The reaction is terminated by adding stop solution, producing a yellow color. The resulting optical density (OD), measured at 450 nm, is directly proportional to the concentration of Anti Liraglutide antibodies present in the samples or standards.

Experimental Design

- An Indirect ELISA was performed using Liraglutide as the capture antibody.
- Standards were prepared using Liraglutide Antibody, Rabbit Polyclonal, AF Purified reference material.
- Assay Concentration Range: 0 - 4000 ng/ml.
- Signal (% absorbance) plotted versus concentration.
- The optimised antigen-coating and detection strategy employed in the Anti-Liraglutide Antibody ELISA ensures highly specific capture of anti-Liraglutide immunoglobulins while minimizing non-specific background arising from endogenous antibodies, circulating GLP-1, or other serum components. This carefully designed assay provides excellent sensitivity and selectivity for the detection of binding anti-drug antibodies, making it well suited for immunogenicity assessment during Liraglutide therapy in both research settings and clinical monitoring studies.

The KRIBIOLISA® Anti-Liraglutide ELISA utilizes an indirect immunoassay format based on the specific interaction between Liraglutide and anti-Liraglutide antibodies present in the sample. Liraglutide are pre-coated onto microwells to act as capture molecules. Patient samples and Liraglutide Antibody, Rabbit Polyclonal, AF Purified standards are added, permitting the drug to bind to the immobilized capture antigen. An Anti-Human

IgG:HRP-conjugated detection antibody is then applied to form a stable immune complex. After washing to remove unbound material, TMB substrate is added, generating a colorimetric signal proportional to the amount of Anti Liraglutide present in the sample. The reaction is stopped using stop solution, and absorbance is measured at 450 nm, providing a reliable quantitative determination of circulating Anti Liraglutide levels.

Validation Parameters and Acceptance Criteria

1. Anti- Liraglutide Values and Recommended ELISA Range

This table summarizes Anti-Liraglutide levels across different therapies and suggested corresponding ELISA working ranges.

Application	Expected Anti-Liraglutide Range (ug/ml)	Recommended ELISA Range (ug/ml)
Baseline / pre-treatment screening (ADA-negative reference)	Not detectable – 0.1	0–1
Early treatment phase (low-titer or transient ADA response)	0.1–1	0–5
Sustained therapy with moderate ADA development (possible PK impact)	1–10	0–20
High-titer or persistent ADA response (potential neutralizing activity)	10–100	0–200

Note: Assay sensitivity in the low ng/mL or equivalent binding-unit range is recommended for the reliable detection of anti-Liraglutide antibodies at baseline and during early or low-titer immunogenic responses. An adequately wide upper quantification range is advised to allow accurate measurement of high-titer anti-drug antibodies that may develop during prolonged or intensified Liraglutide therapy. Such performance characteristics are important for comprehensive immunogenicity assessment, evaluation of potential impacts on drug exposure and efficacy, and long-term monitoring of treatment response.
The KRIBIOLISA® Anti Liraglutide ELISA kit is developed using an assay range of 0 - 4000 ng/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 32000 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 Anti-Liraglutide ELISA are highly specific antibodies and assay components designed to selectively recognize anti-Liraglutide antibodies without cross-reactivity to endogenous immunoglobulins or unrelated circulating antibodies. The assay is optimized to detect antibodies directed against Liraglutide-specific epitopes, including peptide backbone and conformational determinants associated with the acylated GLP-1 analog structure, ensuring high-affinity and selective binding of anti-drug antibodies present in patient samples.

The specificity profile enables accurate discrimination of anti-Liraglutide antibodies in complex biological matrices such as human serum or plasma while minimizing interference from free circulating Liraglutide, endogenous GLP-1, albumin-bound drug complexes, or other incretin-based therapeutics. The assay demonstrates minimal cross-reactivity with structurally related GLP-1 receptor agonists, endogenous peptide hormones, or unrelated therapeutic proteins.

This high degree of molecular specificity ensures reliable detection and quantification of anti-Liraglutide antibodies across a wide range of antibody titers, even in samples with elevated drug levels or metabolic background interference. Consequently, the assay supports robust immunogenicity assessment, pharmacovigilance, and clinical monitoring of immune responses during Liraglutide therapy, provided that antibody binding epitopes remain immunoreactive.

2.2 Selectivity

The Anti-Liraglutide ELISA demonstrates minimal to no cross-reactivity with endogenous human immunoglobulin subclasses, naturally occurring antibodies, or unrelated therapeutic monoclonal antibodies. The assay selectively detects antibodies directed against Liraglutide-specific antigenic determinants and effectively excludes antibodies that do not recognize the unique structural and conformational epitopes of the Liraglutide molecule, including epitopes shared with endogenous GLP-1 or unrelated peptide hormones.

The assay maintains high selectivity in complex biological matrices such as human serum, plasma, or cell-culture supernatants, with negligible interference from free circulating Liraglutide, albumin-bound drug complexes, endogenous GLP-1, other incretin hormones, cytokines, metabolic peptides, heterophilic antibodies, or IgG-binding serum proteins. In addition, minimal cross-reactivity is observed with structurally related GLP-1 receptor agonists, ensuring discrimination of anti-Liraglutide antibodies from immune responses directed against other incretin-based therapies.

This stringent selectivity profile ensures reliable and accurate detection of anti-Liraglutide antibodies without false-positive signals arising from biologically active matrix components or therapeutically related agents, thereby supporting robust immunogenicity assessment and clinical monitoring during Liraglutide therapy.

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 KRIBIOLISA® Anti-Liraglutide ELISA = 20.50 ng/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).

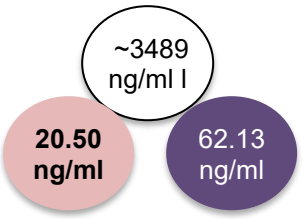
LOQ for KRIBIOLISA® Anti-Liraglutide ELISA – 62.13 ng/ml

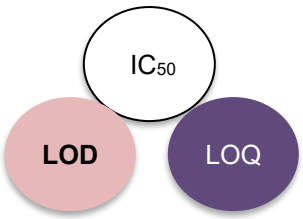
IC_{50} in ELISA (Half Maximal Inhibitory Concentration)
IC_{50} = The concentration of an inhibitor (drug, antibody, compound) required to reduce the signal (e.g., binding, enzymatic activity) by 50% compared to the maximum signal in the assay.

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 KRIBIOLISA® Anti-Liraglutide ELISA = ~3489 ng/ml
Summary:

Parameter	Value (ng/ml)
LOD	20.50 ng/ml
LOQ	62.13 ng/ml
IC_{50}	3489 ng/ml

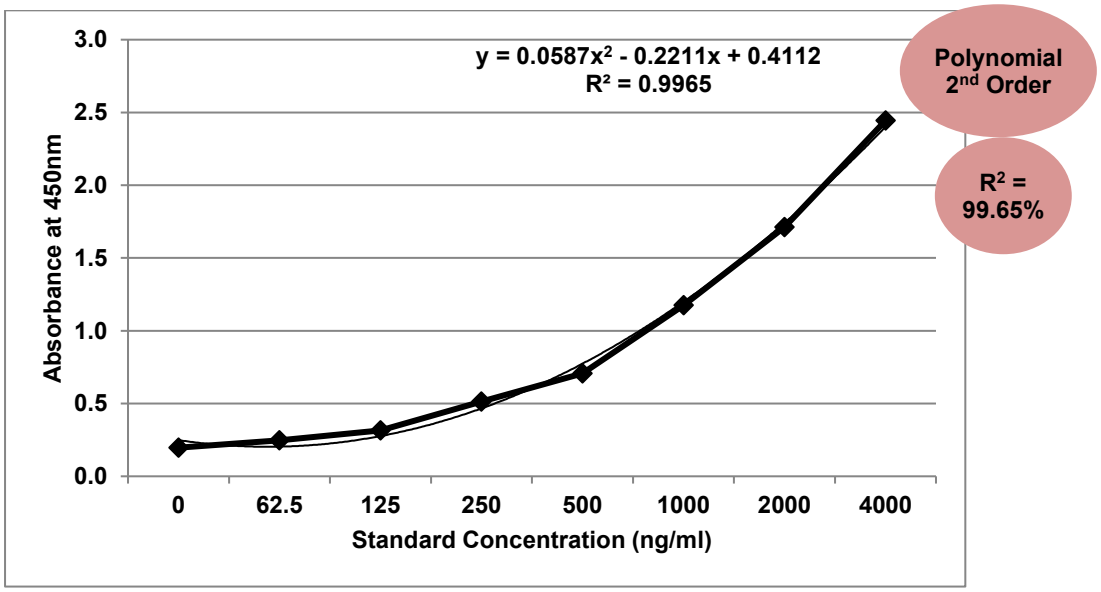


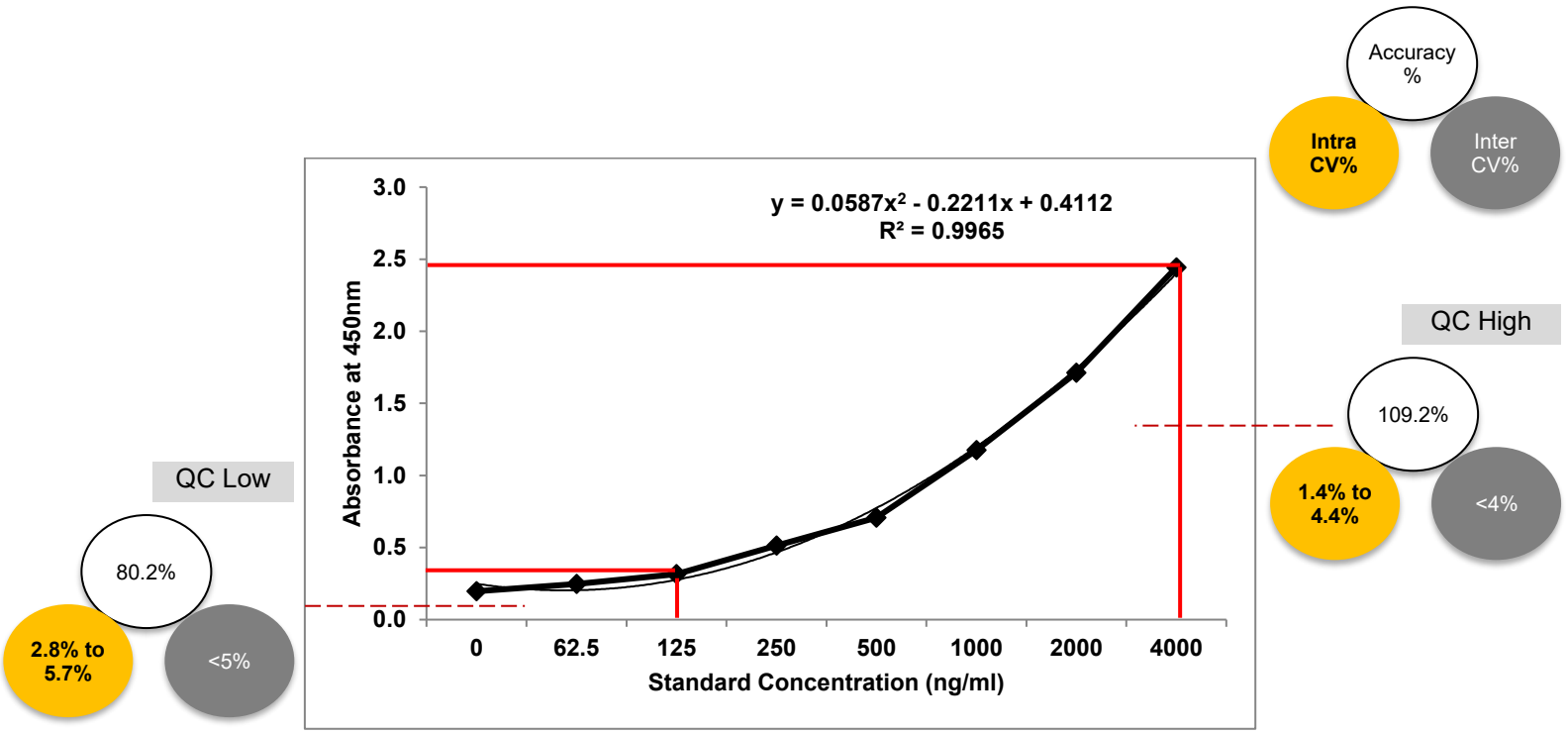


Regulatory Note:
LOD S/N ≥ 3:1, LOQ ≥ 10:1, %CV ≤ 20% **S/N = Signal / Noise Ratio*

3. Linearity and Range

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery
0	0.197	--	--
62.5	0.248	50.8	81.3
125	0.315	105.2	84.2
250	0.513	280.3	112.1
500	0.707	473.1	94.6
1000	1.176	1043.6	104.4
2000	1.713	1954.7	97.7
4000	2.443	4022.5	100.6
Positive Control (3000 ng/ml)	2.322	3577.2	119.2
Low QC control (125 ng/ml)	0.303	100.3	80.2
High QC control (4000 ng/ml)	2.527	4366	109.2





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

Precision was assessed by analyzing three standard concentrations (80 ng/ml, 320 ng/ml, and 2560 ng/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 (ng/ml)	Intra-Assay %CV (Range)	Inter-Assay %CV
80	2.6% to 3%	<3%
320	0.9% to 4.5%	<4%
2560	1.4% to 4.3%	<4%

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 KRIBIOLISA® Anti-Liraglutide ELISA 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

Serial dilutions of a high-concentration sample were prepared at dilutions of 1:2000, 1:4000, 1:8000, 1:16000, 1:32000 and 1:64000 for both human serum and human plasma. Each dilution was assayed using the KRIBIOLISA Anti-Liraglutide ELISA 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 (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:2000	2000	1.672	1872	93.6	93.6
1:4000	1000	1.051	875	87.5	87.5
1:8000	500	0.656	420.3	84.1	84.1
1:16000	250	0.452	224.1	89.6	89.6
1:32000	125	0.327	115.2	92.2	92.2
1:64000	62.5	0.208	19.8	31.6	31.6

B) Human Plasma:

Dilution	Expected Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:2000	2000	1.644	1817	95.2	95.2
1:4000	1000	1.109	951.6	101.8	101.8
1:8000	500	0.669	433.6	96.5	96.5
1:16000	250	0.485	254.3	116.7	116.7
1:32000	125	0.325	113.5	109.9	109.9
1:64000	62.5	0.228	35.2	74.9	74.9

Results:

i. Parallelism is generally maintained across the 1:2000 to 1:32000 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 KRIBIOLISA® Anti Liraglutide ELISA kit was tested for matrix effect on human serum and 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 Anti Liraglutide ELISA without significant loss of accuracy.

7. Matrix Effect Study

Matrix effect was evaluated by comparing the assay performance of standards prepared in:

- Assay buffer (only buffer)
- Assay buffer spiked with human serum (buffer + 1:1000 human serum)
- Assay buffer spiked with human serum (buffer + 1:1000 human plasma)

Samples were tested across the standard curve range (0–4000 ng/ml). Mean absorbance, % Standard Deviation, and % Coefficient of Variation (%CV) were calculated to assess the impact of the serum matrix.

Matrix Effect Study Results

Standard (ng/ml)	Mean Absorbance (Buffer)	Mean Absorbance (Buffer + 1:1000 Human Serum)	% Standard Deviation	% CV
0	0.148	0.197	0.034	19.9
62.5	0.224	0.248	0.017	7.1
125	0.314	0.315	0	0.1
250	0.451	0.513	0.044	9.2
500	0.651	0.707	0.04	5.8
1000	0.903	1.176	0.193	18.6
2000	1.459	1.713	0.18	11.3
4000	2.094	2.443	0.247	10.9

Standard (ng/ml)	Mean Absorbance (Buffer)	Mean Absorbance (Buffer + 1:1000 Human Plasma)	% Standard Deviation	% CV
0	0.148	0.181	0.011	6.1
62.5	0.224	0.224	0.017	7
125	0.314	0.32	0.004	1.2
250	0.451	0.467	0.033	6.7
500	0.651	0.684	0.016	2.3
1000	0.903	1.068	0.076	6.8
2000	1.459	1.712	0.001	0
4000	2.094	2.383	0.043	1.8

Results:

i. Very low %CV across all concentrations.
ii. Minimal shift in absorbance values between buffer-only and buffer + serum and buffer + plasma conditions.
iii. No significant matrix effect observed.

Conclusion:

The KRIBIOLISA® Anti Liraglutide ELISA demonstrates excellent performance in the presence of human serum and plasma. The assay results confirm the absence of significant matrix interference, supporting its reliability for analyzing biological samples.

8. Accelerated Stability Study:

Accelerated stability studies in ELISA are performed to predict the shelf life and long-term stability of an ELISA kit or its individual components by exposing them to elevated stress conditions (typically higher temperatures) for a defined period.

The following table demonstrates the relation of temperature with time point and number of days:

Accelerated Study Day (37 degrees)	Real-Time Equivalent Age (2-8 degree)	Interpretation
Day 0	Present day (0 months)	Initial / release testing
Day 1	26 days (Approx. 1 month)	Early stability checkpoint
Day 4	104 days (Approx. 3.5 months	Short-term stability trend
Day 7	182 days (Approx. 6 months)	Mid-term shelf-life prediction
Day 14	364 days (Approx. 1 year)	One-year shelf-life equivalence

Accelerated Stability Study data:

Standard Concentration (ng/ml)	Absorbance (Day 0)	Absorbance (Day 1)	Absorbance (Day 4)	Absorbance (Day 7)	Absorbance (Day 14)	%CV
0	0.191	0.196	0.189	0.199	0.185	2.8
62.5	0.246	0.260	0.269	0.266	0.250	3.8
125	0.323	0.345	0.319	0.327	0.334	3.2
250	0.475	0.534	0.482	0.448	0.477	6.5
500	0.744	0.792	0.761	0.699	0.751	4.5
1000	1.244	1.335	1.272	1.190	1.287	4.3
2000	1.969	1.951	1.829	1.764	1.913	4.6
4000	2.502	2.698	2.546	2.441	2.443	4.2

Results:

I. %CV is less that 15% across all days.
II. Based on the accelerated stability study results, the Anti Liraglutide ELISA kit demonstrates satisfactory stability and robustness, supporting its viability with an extended shelf life and an assigned expiry of 1 year under recommended storage conditions.

9. Cross Reactivity:

To assess the cross-reactivity and specificity of the KRIBIOLISA Anti Liraglutide ELISA, a comparative evaluation was conducted using Semaglutide and Lixisenatide alongside Anti Liraglutide.

The following table demonstrates Anti Liraglutide standard readings:

Standard Concentration (ng/ml)	Absorbance 1	Absorbance 2	Mean Absorbance	Interpolated Concentration	% Recovery
0	0.199	0.194	0.197	11.5	
62.5	0.253	0.242	0.248	50.8	81.3
125	0.309	0.321	0.315	105.2	84.2
250	0.582	0.445	0.513	280.3	112.1
500	0.722	0.692	0.707	473.1	94.6
1000	1.185	1.168	1.176	1043.6	104.4
2000	1.725	1.701	1.713	1954.7	97.7
4000	2.523	2.364	2.443	4022.5	100.6

The following table demonstrates Semaglutide standard readings:

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Recovery
0	0.171	Below Detection range	#DIV/0!
62.5	0.166	Below Detection range	0.0

125	0.156	Below Detection range	0.0
250	0.150	Below Detection range	0.0
500	0.177	Below Detection range	0.0
1000	0.192	7.8	0.8
2000	0.142	Below Detection range	0.0
4000	0.143	Below Detection range	0.0

Comparison between Anti Liraglutide and Semaglutide Interpolated concentration:

	Anti Liraglutide standard	**Semaglutide**	
Standard Concentration (ng/ml)	**Interpolated Concentration**	**Interpolated Concentration**	**% Cross reactivity**
0	11.5	Below Detection range	Below Detection range
62.5	50.8	Below Detection range	Below Detection range
125	105.2	Below Detection range	Below Detection range
250	280.3	Below Detection range	Below Detection range
500	473.1	Below Detection range	Below Detection range
1000	1043.6	7.8	0.7
2000	1954.7	Below Detection range	Below Detection range
4000	4022.5	Below Detection range	Below Detection range

Results:

The table demonstrates that Anti Liraglutide ELISA has no cross reactivity with Semaglutide.

The following table demonstrates Anti Liraglutide standard readings:

Standard Concentration (ng/ml)	**Absorbance 1**	**Absorbance 2**	**Mean Absorbance**	**Interpolated Concentration**	**% Recovery**
0	0.199	0.194	0.197	11.5	
62.5	0.253	0.242	0.248	50.8	81.3
125	0.309	0.321	0.315	105.2	84.2
250	0.582	0.445	0.513	280.3	112.1
500	0.722	0.692	0.707	473.1	94.6
1000	1.185	1.168	1.176	1043.6	104.4
2000	1.725	1.701	1.713	1954.7	97.7
4000	2.523	2.364	2.443	4022.5	100.6

The following table demonstrates Lixisenatide standard readings:

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Recovery
0	0.163	Below Detection range	#DIV/0!
62.5	0.163	Below Detection range	0
125	0.163	Below Detection range	0
250	0.158	Below Detection range	0
500	0.156	Below Detection range	0
1000	0.16	Below Detection range	0
2000	0.148	Below Detection range	0
4000	0.136	Below Detection range	0

Comparison between Anti Liraglutide and Lixisenatide Interpolated concentration:

	Anti Liraglutide standard	Lixisenatide	
Standard (ng/ml)	Interpolated Concentration	Interpolated Concentration	% Cross reactivity
0	11.5	Below Detection range	0.0
62.5	50.8	Below Detection range	0.0
125	105.2	Below Detection range	0.0
250	280.3	Below Detection range	0.0
500	473.1	Below Detection range	0.0
1000	1043.6	Below Detection range	0.0
2000	1954.7	Below Detection range	0.0
4000	4022.5	Below Detection range	0.0

Results:

The table demonstrates that Anti Liraglutide ELISA has no cross reactivity with Lixisenatide.

10. Sample Handling and Storage Conditions

A) Specimen Collection and Handling:

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20°C.

For Cell Culture Supernatant **–** If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80ºC. Avoid repeated freeze-thaw cycles.

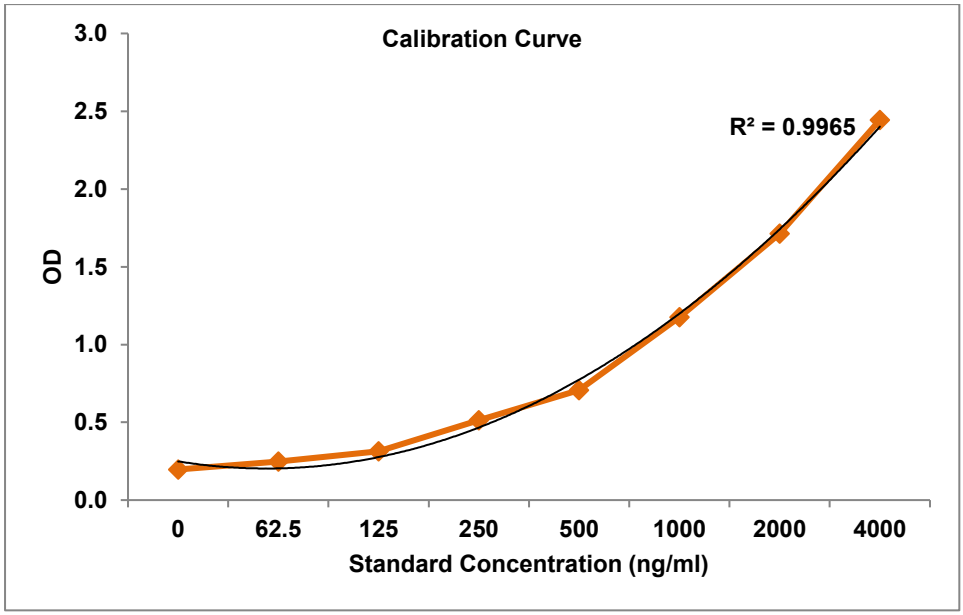
B) Handling / Storage:

- It is advisable to aliquot and store the Anti- Human IgG HRP Conjugate concentrated at -20°C upon receipt. Rest of the kit components should be stored at 2-8°C. Immediately discard any excess working Anti- Human IgG: HRP Conjugate after running your assay.
- All the reagents and wash solutions should be used within 12 months from manufacturing date.
- Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
- The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

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

	1:2000	1:4000	1:8000	1:16000	1:32000	1:64000
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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Kapodistria, K., Tsilibary, E., Kotsopoulou, E., Moustardas, P., & Kitsiou, P. (2018). Liraglutide, a human glucagon-like peptide-1 analogue, stimulates AKT-dependent survival signalling and inhibits pancreatic β-cell apoptosis. *Journal of Cellular and Molecular Medicine*, *22*(6), 2970–2980. https://doi.org/10.1111/jcmm.13259