VALIDATION OF KRIBIOLISA™ Anti-Liraglutide ELISA (Catalog No: KBI9040) AS PER FDA GUIDELINES FOR BIOANALYTICAL METHOD VALIDATION

This validation protocol has been adopted in line with the Methodology and Analytical Procedures Guideline recommended by FDA (guidelines May 2018).

Document History

First Codification	History	Date
Version#1	**KRIBIOLISA™ Anti-Liraglutide ELISA (CATALOG NO. KBI9040)**	30th June 2023

Approved: Quality Control	Approved: Product Development
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KRISHGEN BIOSYSTEMS

Introduction

This document presents a discussion of the characteristics of our KRIBIOLISA™ Anti-Liraglutide Elisa Kit (Catalog No. KBI9040). This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays. 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 antibodies to Liraglutide**.**

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

- **Limit Of Detection**
- **Specificity**
- **Precision**
- **Cross Reactivity**
- **Matrix Effect / Recovery**
- **Validation kit lot specific details**
- **Lot-to-Lot Consistency**

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 sales@krishgen.com.

Background

Liraglutide (NN2211) is a derivative of a human incretin (metabolic hormone), glucagon-like peptide-1 (GLP-1) that is used as a long-acting glucagon-like peptide-1 receptor agonist, binding to the same receptors as does the endogenous metabolic hormone GLP-1 that stimulates insulin secretion. Marketed under the brand name Victoza, it is an injectable drug developed by Novo Nordisk for the treatment of type 2 diabetes. In 2015, Novo Nordisk began marketing a separate strength in the U.S. and E.U. under the brand name Saxenda as a treatment for adults who are obese or overweight with at least one weight-related comorbid condition.

Validation Information

1. Sensitivity:

Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be ~ 8 ng/ml. Each lot is optimized to ensure high signal, low background, and the consistent sensitivity.

2. Specificity:

The kit uses a capture peptide immobilized on the microplate which is a synthetic liraglutide peptide bearing CAS No.: 204656-20-2 and with the molecular formula: $C_{172}H_{265}N_{43}O_{51}$ and molecular weight: 3751.26. The details of the Sequence Shortening are: HAEGTFTSDVSSYL-{N6-[N-(1-oxohexadecyl)-L-γ-Etamyl]-Glu}-GQAAKEFIAWLVRGRG. The standards used in the kit is a GLP-1 polyclonal antibody having 100% cross reactivity against liraglutide. The standard antibody immunogen range is 1-31/31.

3. Precision:

Inter/Intra Assay Precision

Precision is defined as the percent coefficient of variation (%CV) i.e., standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (10 ng/ml), medium (80 ng/ml) and high (640 ng/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay % CV	Inter Assay % CV
Low	<10%	<10%
Medium	<5%	<5%
High	<5%	<5%

4. Traceability and Stability:

4.1 Traceability:

The standards used in this kit have been calibrated against commercially sourced recombinant anti-liraglutide antibodies.

4.2 Stability:

Shelf-Life Stability: An accelerated stability study set the shelf-life stability of KRIBIOLISA™ Anti-Liraglutide ELISA was performed, and a shelf life of 12 months was assigned to the kit.

5. Serum/Plasma Spike Recovery:

Normal human serum was used to spike at 640ng/ml (being the highest standard) to check the recovery at different dilutions to observe optimal recoveries. It was seen that 1:1000 serum dilution reported the most optimal recoveries.

Serum and plasma spiked at 640 ng/ml with Anti-Liraglutide standard, and dilution done for analysis at 1:100 and 1:1000.

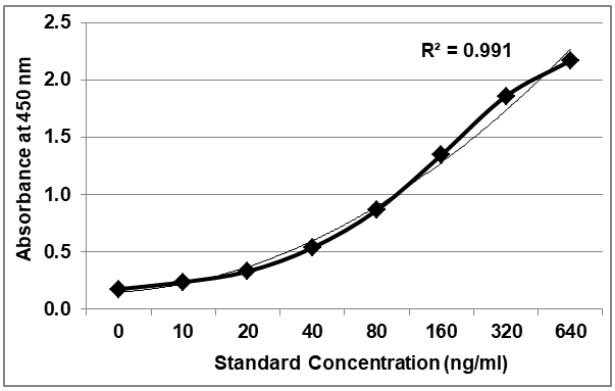
Standard Diluent	Standard (ng/ml)	Mean Abs @ 450nm
1:100 Human Serum	0	0.740
	640	4.000
1:1000 Human Serum	0	0.114
	640	2.492

Standard Diluent	Standard (ng/ml)	Mean Abs @ 450nm
1:100 Human Plasma	0	0.462
	640	4.000
1:1000 Human Plasma	0	0.128
	640	2.483

6. Validation Kit Lot Specific Details:

Standard Concentration (ng/ml)	Absorbance A	Absorbance B	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.163	0.191	0.177	--	--
10	0.238	0.239	0.238	9.3	93.2
20	0.326	0.338	0.332	19.9	99.6
40	0.523	0.552	0.538	41.5	103.9
80	0.857	0.877	0.867	79.3	99.1
160	1.309	1.391	1.350	157.3	98.3
320	1.822	1.903	1.863	329.6	103.0
640	2.133	2.212	2.172	626.7	97.9

Typical Graph:



7. Lot-to-Lot Consistency

All lots are tested to ensure low background, a linear standard curve, consistent assay sensitivity, and a broad dynamic standard curve range. Consistent standard curve O.D.s and control values ensure that sample data is comparable over time.