VALIDATION OF KRIBIOLISA™ Semaglutide (Ozempic™) ELISA (Catalog No: KBI5030) 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™ Semaglutide (Ozempic™) ELISA (CATALOG NO. KBI5030)**	30th June 2023

Approved: Quality Control	Approved: Product Development
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Introduction

This document presents a discussion of the characteristics of our **KRIBIOLISA™ Semaglutide (Ozempic™) ELISA (Catalog No. KBI5030).** 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 Semaglutide.

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

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

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

Semaglutide (trade name Ozempic) is a pharmaceutical drug in development by a Danish company Novo Nordisk for the treatment of type 2 diabetes. It is marketed by the name Ozempic. As a glucagon-like peptide-1 receptor agonist, it lowers the blood sugar level by increasing the production of insulin. It was discovered in 2012, by a team of researchers at Novo Nordisk as a longer-acting alternative to Liraglutide. Clinical trials were started in 2015, and phase 3 was completed in 2016. FDA approval was applied in December 2016, and in October 2017 FDA Advisory Committee voted 16-0 in favor. It can be used as both injection-type and an oral-type drug.

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, in duplicate, were evaluated and the LOD was found to be less than 50 ng/ml. Each lot is optimized to ensure high signal, low background, and the consistent sensitivity.

2. Specificity:

The capture antibody used in the kit is a GLP-1 monoclonal antibody which reacts with all forms of GLP-1, including precursor. The immunogen used is a synthetic peptide corresponding to Human GLP-1 aa 50-150. The immunogen sequence corresponds to Glucagon-like peptide 1 (7-36), one of the chains formed when cleaved.

The standard / calibrator used in the kit is Semaglutide with the molecular formula: C187H291N45O59•XC2H4O2 and molecular weight 4113.6. and is a crystal powder. The standard / calibrator used was certified against commercially available Ozempic™.

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 (50 ng/ml), medium (1000 ng/ml) and high (4000 ng/ml) concentrations, run in duplicates. 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%

Intra-Assay Precision –

Intra-assay validation shows the reproducibility between wells within an assay plate. Data resulting from intra-assay validation helps ensure that samples run in different wells of the plate will give comparable results. 5 replicates each for three samples in the same assay were run and the resulting low %CV for each sample indicates good reproducibility within the assay. Samples with known semaglutide concentration were assayed in replicates of 5 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/ml)	55	1300	3500
SD	0.10	0.32	1.43
CV (%)	4.81	8.43	7.49

SD = standard deviation, CV = coefficient of variation.

Inter-Assay Precision –

Inter-assay precision shows the reproducibility between assays done on different days. Data generated and reported for inter-assay precision was generated with three samples, 10 times over multiple days. The results show CVs of less than a 10%, thus demonstrating good reproducibility between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/ml)	55	1300	3500
SD	1.56	3.21	2.12
CV (%)	5.65	8.94	7.32

SD = standard deviation, CV = coefficient of variation.

4. Cross-Reactivity

Marker	% Cross Reactivity
Liraglutide	100-120%
endogenous GLP-1	<0.1%

5. Serum/Plasma Spike Recovery:

Normal human serum was used to spike at 4000 ng/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.

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.

6. Validation Kit Specific Details:

Standard Concentration (ng/ml)	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	2.791	--	--
50	2.413	57.7	115.4
100	2.241	124.0	124.0
500	1.881	360.5	72.1
1000	1.332	1077.1	107.7
2000	0.867	2227.4	111.4
3000	0.647	3045.8	101.5
4000	0.493	3765.5	94.1

Typical Graph: