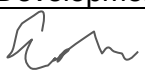
VALIDATION OF KRIBIOLISA™ Anti-Semaglutide(Ozempic™) ELISA(CATALOG NO. KBI9030) AS PER FDA GUIDELINES FOR BIOANALYTICAL METHOD VALIDATION

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-Semaglutide (Ozempic™) ELISA(CATALOG NO. KBI9030)	

Approved Quality Control	Approved Product Development
	
Ankita G	Atul G

Introduction

This document presents a discussion of the characteristics of our **KRIBIOLISA™ Anti-Semaglutide(Ozempic™) ELISA(CATALOG NO. KBI9030).** 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 Semaglutide**.**

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

- **Sensitivity**
- **Specificity**
- **Precision**
- **Traceability / Stability**
- **Recovery**
- **Validation kit 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 or oral-type drug.

Validation

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 less than 125 ng/ml. Each lot is optimized to ensure high signal, low background, and the consistent sensitivity.

2. Specificity

The immobilized capture is a Semaglutide peptide which is synthetically prepared having a molecular formula C187H291N45O59 • XC2H4O2 and molecular weight 4113.6. The peptide is synthesized as per known amino sequences. The standard used in the kit is a polyclonal antibody with 100% cross reactivity to Semaglutide.

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 (125 ng/ml), medium (1000 ng/ml) and high (8000 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-semaglutide antibodies.

4.2 Stability:

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

5. Serum/Plasma Spike Recovery:

Serum and plasma were spiked at 2000ng/ml with Anti-Semaglutide(Ozempic™) standard with dilution for analysis at 1:10, 1:100 and 1:1000. Results were observed as below, with 1:1000 dilution reported the most optimal recoveries.

Standard Diluent	Standard (ng/ml)	Mean Abs @ 450nm
1:10 Human Serum	0	4.000
	2000	4.000
1:100 Human Serum	0	2.283
	2000	4.000
1:1000 Human Serum	0	0.864
	2000	4.000

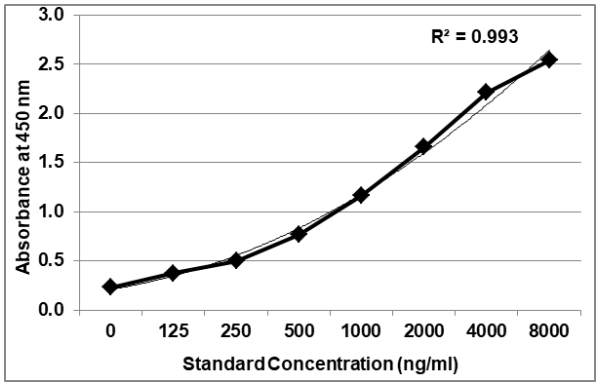
Standard Diluent	Standard (ng/ml)	Mean Abs @ 450nm
1:10 Human Plasma	0	4.000
	2000	4.000
1:100 Human Plasma	0	2.075
	2000	4.000
1:1000 Human Plasma	0	0.755
	2000	4.000

6. Validation Kit Specific Details:

Typical Data

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.234	--	--
125	0.377	132.1	105.7
250	0.501	245.5	98.2
500	0.767	508.8	101.8
1000	1.164	1001.0	100.1
2000	1.658	1940.7	97.0
4000	2.213	4186.3	104.7
8000	2.544	7778.2	97.2

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.