# KRIBIOLISA™ Neutralizing Antibodies to Semaglutide (OZEMPIC™) ELISA

**REF** : KBN1930

Ver 1.3

**RUO**

Enzyme Immunoassay for the Qualitative Detection of Neutralizing Antibodies against Semaglutide in human serum

| | | | |
|---|---|---|---|
| **RUO** | **For Research Use Only** | **REF** | **Catalog Number** |
|  | **Store At** | **LOT** | **Batch Code** |
|  | **Manufactured By** |  | **Biological Risk** |
|  | **Expiry Date** |  | **Consult Operating Instructions** |

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**REF** KBN1930 Σ 96 tests

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**Introduction:**

The KRIBIOLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum and cell culture supernatant as validated with the kit. The kit employs a blocking ELISA technique which engages the blocking pathway to estimate the neutralizing antibodies.

Semaglutide is a glucagon-like peptide 1 (GLP-1) analog used to manage type 2 diabetes along with lifestyle changes, such as dietary restrictions and increased physical activity. GLP-1, a major incretin hormone in humans, acts by numerous mechanisms like augmented insulin secretion (glucose-dependent), inhibition of glucagon release and suppressed hepatic gluconeogenesis. It also causes delayed gastric emptying, reduced appetite and energy intake. Reduction of HbA1c level along with body weight without any risk of hypoglycaemia, provides it a special status for the treatment of obese type 2 diabetic patients.

Semaglutide uses three strategies to improve the residence time of the peptide. First, stearic diacid is used instead of palmitic acid to increase the molecule's affinity for albumin. Second, an extended spacer consisting of two 8-amino3,6- dioxaoctanoic acid (ADO) moieties and a glutamic acid residue further improves albumin binding stability. Third, the peptide's second residue is mutated to aminoisobutyric acid to confer greater DPP-IV resistance. These modifications result in a half-life of 165 hours in humans, making it suitable for once-weekly administration. The fatty di-acid chain attached to the GLP-1R agonist provides semaglutide with a higher affinity for albumin.

Immunogenicity is also a critical concern with this class of therapeutics. Prolonged exposure to the drug could initiate the development of neutralizing antidrug antibodies, which reduces safety and efficacy. The production of ADAs is generally a treatment risk with therapeutic peptides and can lead to an altered pharmacokinetic (PK) profile, which in turn may impact the safety and efficacy of therapeutic peptides (Wang, L., Wang, N., Zhang, W. et al. Therapeutic peptides: current applications and future directions.). ADAs can be binding, leading to minimal or no impact; clearing, leading to impact through an altered PK profile; sustaining, leading to a prolonged exposure that may change the efficacy and/or safety; or neutralizing, leading to reduced pharmacological activity of the therapeutic antibody (Garcês and Demengeot 2018; Bloem et al. 2017).

Regulatory agencies prefer cell-based assays because they rely on cellular responses to NAb-mediated inhibition of therapeutic antibodies (Wu et al. 2016). Because of their mode of detection, these assays have been considered more biologically relevant than non–cell-based assays (Bloem et al. 2017; Liao et al. 2012; Cong et al. 2015; Jolicoeur and Tacey 2012). However, cell-based assays are labor intensive and highly variable and exhibit low serum tolerance and poor drug tolerance (Bloem et al. 2017; Liao et al. 2012; Cong et al. 2015; Jolicoeur and Tacey 2012; Wu et al. 2016). In contrast to cell-based assays, non–cell-based assays often rely on binding of the drug and target for signal detection and quantitation. Competitive ligand-binding assays, used to characterize NAbs, have been shown to provide higher sensitivity, a wider dynamic range, increased precision, and better matrix tolerance than cell-based assays (Wu et al. 2018). Although regulatory agencies generally prefer cell-based assays, the United States Food and Drug Administration and the European Medicines Agency recognize both cell-based and non–cell-based competitive ligand-binding assays as valid measurements of NAbs in some cases (Jolicoeur and Tacey 2012; Wu et al. 2016). The selection of either cell-based or non–cell-based assays is driven by several different variables, including therapeutic mechanism of action (MOA), assay performance characteristics, and risk of immunogenicity, with therapeutic MOA being the primary determinant (Wu et al. 2016).

Semaglutide binds to its ligand GLP-1R which intiaties a cascade that involves activation of membrane bound Adenyl Cyclase (AC) and consequent production of cyclic adenosine monophosphate (cAMP). Downstream of cAMP formation, several signal transduction pathways can be initiated, which generally require activation of either one or both of the cellular cAMP effectors, Protein kinase A (PKA) and exchange protein directly activated by cAMP (EPAC).

The KRIBIOLISA Neutralizing Antibodies to Semaglutide is a competitive ligand-binding assay that is specific for the detection of anti-Semaglutide NAbs in human serum. The assay involves the Nab-Semaglutide preventing the binding of Semglutide to GLP-1R. The kit is validated for assay precision, sensitivity, hook effect, selectivity, robustness, stability, and system suitability.

**Intended Use:**

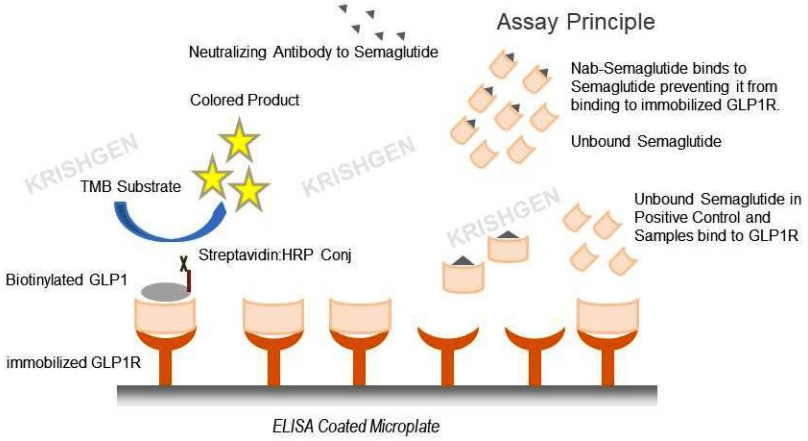
The KRIBIOLISA™ Neutralizing Antibodies to Semaglutide (OZEMPIC™) ELISA kit is used as an analytical tool for the qualitative detection of neutralizing antibodies against Semaglutide in serum or plasma.

**Principle:**

The method employs a two-step competitive inhibition ELISA technique.
The first step involves the antibody-protein binding between the neutralizing antibody and Semgalutide.
The second step involves competiton between the unbound Semaglutide and Biotin:Anti-GLP-1 Antibody to bind to GLP-1R.

Samples and controls are pipetted in a blank microtitre plate and incubated with Semaglutide. Neutralizing Antibodies to Semaglutide present in the samples / positive control will bind to Semaglutide. This complex of bound and unbound Semaglutide is then incubated with immobilized GLP-1R in a second plate. The unbound Semaglutide present will bind to GLP-1R.

After washing, the detection conjugate of Biotin:Anti-GLP-1 Antibody is added. Post washing Streptavidin:HRP is pipetted. After incubation and washing, a substrate solution (TMB) is added to the microwells. Post incubation, color develops inversely proportionate to the amount of unbound Semaglutide in the sample which inhibits GLP-1 to bind to the immobilized GLP-1R. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.




**PRINCIPLE OF THE KRIBIOLISA™ NEUTRALIZING ANTIBODY TO SEMAGLUTIDE (OZEMPIC™/WEGOVY™) ELISA**

**Materials Provided:**

| Part | Description | Qty |
|---|---|---|
| GLP-1R Coated Microtiter Plate | 96 well polystyrene microplate (12 strips of 8 wells) coated with GLP-1R | 1 x 96 wells |
| Blank Microtiter Plate | 96 well polystyrene uncoated microplate (12 strips of 8 wells) | 1 x 96 wells |
| Neutralizing Anti-Semaglutide Positive Control | Neutralizing Anti-Semaglutide Positive Control (lyophilized) | 2 vials |
| Semaglutide | Semaglutide Research Grade (lyophilized) | 6 vials |
| Semaglutide Diluent | Buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane | 12 ml |
| Biotin:Anti-GLP-1 | Biotin:Anti-GLP-1 prepared in buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane. | 12 ml |
| Streptavidin:HRP concentrated | Streptavidin:HRP Conjugate concentrated | 1 vial |
| Strep HRP Diluent | Buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane. | 12 ml |
| (1X) Sample Diluent | Buffered protein base with preservative thiomersol < 0.01% | 1 x 50 ml |
| (1X) Control Diluent | Buffered protein base with preservative thiomersol < 0.01% with 1:10 dilution human serum | 10 ml |
| (20X) Wash Buffer | 20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time. | 25 ml |
| TMB Substrate | Stabilized Chromogen | 12 ml |
| Stop Solution | 0.73M Phosphoric Acid | 12 ml |
| Instruction Manual | | 1 no |

**Materials to be provided by the End-User:**

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

**Handling/Storage:**

1. Store main kit components at recommend temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

**Health Hazard Warnings:**

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.

**Sample Preparation and Storage:**

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

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.

**Samples should be diluted 1:10 (v/v) for optimal recovery, (for example 1 ul sample + 9 ul sample diluent) prior to assay. In cases where matrix interferences is under or over observed, the samples may be diluted less or more respectively with Sample Diluent accordingly.**

The samples may be kept at 2 - 8°C for up to three days. For long-term storage please store at -20°C.
***Note:*** *Grossly hemolyzed samples are not suitable for use in this assay.*

**Preparation before Use:**

Allow serum or plasma samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

In cases where matrix interferences is under or over observed, the samples may be diluted with Sample Diluent accordingly.

The samples may be kept at 2 - 8°C for up to three days. For long-term storage please store at -20°C.

***Note:*** *Grossly hemolyzed samples are not suitable for use in this assay.*

**Reagent Preparation (all reagents should be diluted immediately prior to use):**

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. **Reconstitute the lyophilized Neutralizing Anti-Semaglutide Positive Controls in 125 ul Control Diluent.**
4. **Control diluent is the Neutralizing Anti-Semaglutide Negative Control.**
5. **Reconstitute the lyophilized Semaglutide in 2 ml Semaglutide Diluent.**

6. **Preparation of Working Streptavidin:HRP - Refer to the Reagent Preparation sheet attached with the IFU and COA (enclosed in the kit).**
7. To make **Wash Buffer (1X)**; dilute **25 ml** of **20X Wash Buffer** in **475 ml of DI water**.

**Procedural Notes:**

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. Avoid assay of Samples containing sodium azide ($NaN_3$), as it could destroy the HRP activity resulting in under-estimation of the amount of bound Nab Semaglutide.
3. It is recommended that the Standards and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Standards and Samples.

**Assay Procedure:**

**1) Neutralization Reaction**

1. Pipette **100 ul** of prepared **Positive / Negative Control** in duplicate to the respective wells in the blank microplate.

2. Pipette **100 ul** of the diluted **Samples solution** into the respective wells in the blank microplate.

3. Pipette **100 ul** of the prepared **Semaglutide** into all the wells in the blank microplate.

4. Seal the plate and **incubate** for **60 minutes** at **Room Temperature (18-25°C).**

**2) Binding Reaction**

1. Pipette **100 ul** of the **Positive / Negative Control Solution Complex** into the respective wells of the GLP-1R coated microplate from the neutralization reaction plate.

2. Pipette **100 ul** of the diluted **Samples Solution Complex** into the respective wells of the GLP-1R coated microplate from the neutralization reaction plate.

3. Seal plate and **incubate** for **90 minutes** at **Room Temperature (18-25°C)**.

4. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

5. Pipette **100 ul** of **Biotin:Anti-GLP-1** to all the wells.

6. Seal plate and incubate for **90 minutes** at **Room Temperature (18-25°C)**.

7. Aspirate and wash plate 4 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

8. Pipette 100 ul of diluted **Working Streptavidin:HRP Conjugate** to all the wells.

9. Seal plate and incubate for **60 minutes at Room Temperature (18-25°C).**

10. Aspirate and wash plate 4 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

11. Pipette **100 ul** of **TMB Substrate** solution to all the wells.

12. **Incubate** in the dark for **30 minutes** at **Room Temperature**.

13. Stop reaction by adding **100 ul** of **Stop Solution** to each well.

14. **Read Absorbance** at 450 nm within 30 minutes of stopping reaction.

**Qualitative Interpretation:**

**Calculation for Cut Off Values -**
Read the sample and positive control wells on microtitre plate reader at 450nm.

The OD (Optical Density) of NC (Negative Control) in duplicate should be used for calculating the mean and standard deviation. This is the $Negative_{mean}$.

The Cut-Off for Negative Samples is equal to a value greater than ($Negative_{mean}$ + 2*Standard Deviation).

***Formula:***
***Negative Sample Value = OD > (*$Negative_{mean}$ *+ 2*SD)***

*Typical example –*

| *Sample Type* | *Absorbance #1* | *Absorbance #2* | *Mean* |
|---|---|---|---|
| *Negative* | *1.108* | *1.018* | *1.063* |

Therefore, Cut-Off = Mean + 2*SD
= 1.063 + (2*0.064)
= 1.063 + 0.128
= 1.191

**Interpretation of Results:**

| | |
|---|---|
| **Positive NAb-Semaglutide Samples *** | **< 0.5 *** |
| **Negative Nab-Semaglutide Samples *** | **=>0.5*** |

** The cut-off value is based on validation using recombinant antibodies in the assay. Users may set up their own cut-off values based on different patient serum panels from different co-existent diseases, geographic locations or ethnic backgrounds.*

Explanation of Results Interpetation:

i) If Neutralizing Antibody is present in the sample, it will bind to (neutralize) Semaglutide. Semaglutide will not be able to then inhibit the binding of Anti-GLP-1 to GLP-1r coated well. This in turn will lead to a higher absorbance value as more Anti-GLP-1 will bind to GLP-1r.
ii) if Neutralizing Antibody is not present in the sample, it will lead to no binding (neutralizing) of Semaglutide. Semaglutide will then inhibit the binding of Anti-GLP-1 to GLP-1r coated well. This in turn will lead to a lower absorbance value as less Anti-GLP-1 will be bind to GLP-1r.

**Limitation of the Procedure:**

This ELISA test is designed for qualitative and/or quantitative detection of the neutralizing antibodies to Semaglutide only.

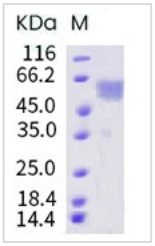
**Performance Characteristics:**

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory.

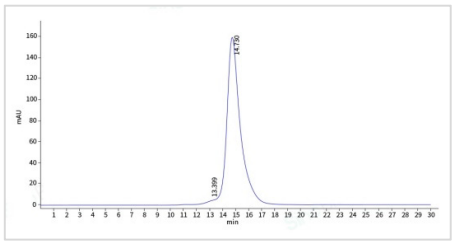
This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results.

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.

**Specificity of the Immobilized GLP-1R:**
The GLP-1R protein construct used a DNA sequence encoding the human GLP1R (NP_002053.3) (Met1-Tyr145) and was expressed with the Fc region of human IgG1 at the C-terminus.




Gel Image of the Coat/Capture Protein



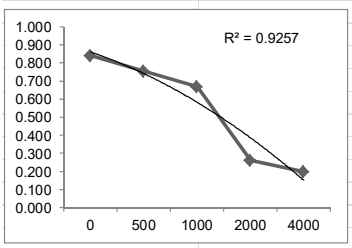
Chromatogram of the Coat/Capture Protein

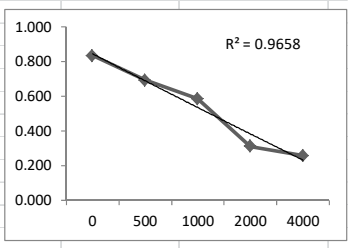
**Specificity of Semaglutide:**
Semaglutide biosimilar research grade is a peptide with molecular formula as C187H291N45O59 • XC2H4O2 and molecular weight of 4113.6. The purity of the semaglutide peptide is ≥95%

**Specificity of the Neutralizing Antibody (Positive Control):**
The Neutralizing Anti-Semaglutide monoclonal antibody binds to Semaglutide and is offered in a lyophilized form.

**Sensitivity of the Neutralizing Antibody (Positive Control):**
Three samples sets were run using Semagludie biosimilar at a concentration of 500 ng/ml. The neutralizing antibody was run at different concentrations spiked in normal human serum. It was oberserved that the assay had a sensitivity of ~400 ng/ml. The assay was run using different concentrations to observe the effects of increased concentrations of the neutralizing antibody.

| NA Concentration (ng/ml) | Mean Absorbance at 450nm | Semaglutide concentration (ng/ml) |
|---|---|---|
| 0 | 0.841 | **500 ng/ml** |
| 500 | 0.754 | |
| 1000 | 0.668 | |
| 2000 | 0.261 | |
| 4000 | 0.200 | |
| 0 | 0.835 | **1000 ng/ml** |
| 500 | 0.694 | |
| 1000 | 0.585 | |
| 2000 | 0.312 | |
| 4000 | 0.259 | |




**Assay Range:**
Qualitative

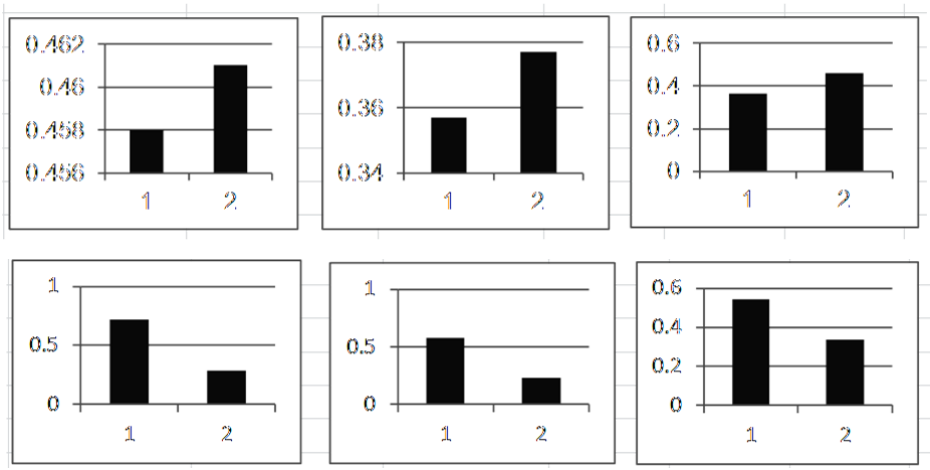
**Precision:**
Intra-Assay: CV<12%
Inter-Assay: CV<18%

**Validation Experiment Done Using Varying Semaglutide (biosimilar) concentrations spiked in normal human serum.**

Using the assay kit, samples were prepared spiking semaglutide biosimilar at varying concentrations as per the table below and the neutralizing antibody was assay to observe the effects of semaglutide present in the sample on the neutralizing antibody. The matrix used to prepare the samples was normal human serum. The samples were run in duplicate.

| NA Concentration (ng/ml) | Mean Absorbance at 450nm | Semaglutide (biosimlar) concentration (ng/ml) |
|---|---|---|
| 0 | 0.458 | 100 ng/ml |
| 2000 | 0.461 | |
| 0 | 0.357 | 200 ng/ml |
| 2000 | 0.377 | |
| 0 | 0.365 | 400 ng/ml |
| 2000 | 0.459 | |
| 0 | **0.723** | 500 ng/ml |
| 2000 | **0.282** | |
| 0 | **0.577** | 1000 ng/ml |
| 2000 | **0.229** | |
| 0 | **0.542** | 2000 ng/ml |
| 2000 | **0.334** | |

** NA = neutralizing antibody to Semaglutide*




It was observed that using neutralizing antibody concentrations of 2000 ng/ml and semaglutide concentrations below 500 ng/ml in spiked normal human serum samples were not recognized. We concluded that at concentrations lower than 500 ng/ml, there is less free semaglutide available to inhibit the binding of GLP1-1R/GLP1R to Anti-GLP-1. Similary beyond 2000 ng/ml of Semaglutide in the sample, the saturation levels were achieved and the diferential signal (absorbance values) between the negative blank and the spiked sample (2000 ng/ml) was minimal.

**Validation Experiment Done Using Varying Ozempic™ Injection concentrations spiked in normal human serum.**

Using the assay kit, samples were prepared spiking semaglutide biosimilar at varying concentrations as per the table below and the neutralizing antibody was assay to observe the effects of semaglutide present in the sample on the neutralizing antibody. The matrix used to prepare the samples was normal human serum. The samples were run in duplicate

| NA Concentration (ng/ml) | Mean Absorbance at 450nm | Ozempic™ concentration (ng/ml) |
|---|---|---|
| 0 | 0.871 | **500 ng/ml** |
| 125 | 0.782 | |
| 250 | 0.658 | |
| 500 | 0.514 | |
| 1000 | 0.458 | |
| 2000 | 0.385 | |
| 4000 | 0.315 | |

** NA = neutralzing antibody to Semalgutide (Ozempic)*

It was concluded that the assay sensitivity when tested with Ozempic Injection was ~120 ng/ml.

**Validation Experiment Done Using Different Antibodies to distinguish the Neutralizing Antibody from the Blocking Antibodies**

The assay uses Anti-GLP-1 specific to bind to GLP1R. Binding antibodies present in the spiked sample do not inhibit the binding of Semalutide to GLP1R. We tested two such antibodies and observed that at similar concentration of 2000 ng/ml these antibodies did not inhibit the binding of Semaglutide to GLP1r.

The antibodies were spiked in normal human serum. The assay was run as per the protocol and the results observed. We concluded these antibodies did not neutralize and hence the absorbances of the blank and antibody concentration of 2000 ng/ml in the presence of Semaglutide (500 ng/ml concentration) led to an increased absorbance value.

| Binding Antibody | Antibody Concentration (ng/ml) | Mean Absorbance at 450nm |
|---|---|---|
| **BA1** | 0 | 0.412 |
| | 2000 | 0.738 |
| **BA2** | 0 | 0.248 |
| | 2000 | 0.469 |

*BA1 and BA2 are commercial antibodies procured as Anti-GLP-1.

**Validation Experiment Done Assuming No Free Semaglutide is there or No Semglutide in the Assay and the Binding Characteristics of Anti-GLP-1 to GLP-1R.**

The assay uses Anti-GLP1 specific to bind to GLP-1R. To ensure that complete free semaglutide is available to bind to GLP1R, the addition of Anti-GLP-1 is done as a two step procedure with an incubation and wash step in between. This ensures that the assay does not produce false results due to the direct binding of Anti-GLP-1 to GLP-1R with no Semglutide available. This could be because the available Semaglutide at a concentration of 500 ng/ml is completely neutralized due to the presence of enough concentration of neutralizing antibodies.

The antibodies were spiked in normal human serum. The assay was run as per the protocol and the results observed. We concluded these antibodies did not neutralize and hence the absorbances of the blank and antibody concentration of 2000 ng/ml and 4000 ng/ml in the presence of no Semaglutide (0 ng/ml concentration).

| NA Concentration (ng/ml) | Absorbance | Semaglutide concentration (ng/ml) |
|---|---|---|
| 0 | 0.602 | **0 ng/ml** |
| 2000 | 0.550 | |
| 4000 | 0.468 | |

**Dilutional Linearity to observe the Matrix Effect on the Assay**

ELISA linearity-of-dilution results for samples using the kit. The best sample diluent was chosen from the previous spike-and-recovery experiments and three dilutions were made for each sample. Observed values were assessed relative to the assay results produced.

It was concluded that the neutralizing antibody sample diluted at 1:10 gave the most optimal results and dilutions beyond 1:100 were not detectable.

| Neutralizing Antibody Sample | Antibody Concentration (ng/ml) | Mean Absorbance at 450nm |
|---|---|---|
| **NA + 1:10 Human serum** | **0** | **0.631** |
| | **2000** | **0.551** |
| **NA + 1:100 Human serum** | 0 | 0.547 |
| | 2000 | 0.440 |
| **NA + 1:1000 Human serum** | 0 | 0.289 |
| | 2000 | 0.618 |

**Note:**
For researchers and clinical companies looking to use the kit for demonstration and estimation of their own developed antibody to Semaglutide with their own Semaglutide, it is recommended to first run similarity assay using the kit provided Semagalutide. Subsequently, you may use your internal Semaglutide in the assay once the parameters are established.

In case the demonstrated variance is beyond the defined quality limits when using your own Semaglutide, we would recommend to use the kit provided Semaglutide only.

In case the recoveries obtained are not as per the desired results, please connect with us (email: sales1@krishgen.com) to help you optimize the assay using our own differently formulated diluents to best optimize on the kit.

**Safety Precautions:**

- **This kit is For Research Use Only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

## Typical Example of a Work List

| Well # | Contents | Absorbance at 450nm | Mean Absorbance |
|---|---|---|---|
| 1A<br>2A | Negative Control<br>Negative Control | | |
| 1B<br>2B | Postive Control<br>Positive Control | | |
| 1C<br>2C | Sample<br>Sample | | |
| 1D<br>2D | Sample<br>Sample | | |
| 1E<br>2E | Sample<br>Sample | | |
| 1F<br>2F | Sample<br>Sample | | |
| 1G<br>2G | Sample<br>Sample | | |
| 1H<br>2H | Sample<br>Sample | | |
| 3A<br>4A | Sample<br>Sample | | |
| 3B<br>4B | Sample<br>Sample | | |
| 3C<br>4C | Sample<br>Sample | | |

## LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems. 2024

***THANK YOU FOR USING KRISHGEN PRODUCT!***



**KRISHGEN BIOSYSTEMS** | OUR REAGENTS | YOUR RESEARCH |
**Ozempic is the registered trademark of NovoNordisk, Denmark.**

## SYMBOLS KEY

| | |
|---|---|
| COATED \| MTP | Coated Microtiter Plate (12x8 wells) |
| UNCOATED \| MTP | Uncoated Microtiter Plate (12x8 wells) |
| PC | Positive Control |
| SEMA \| DIL | Semaglutide Diluent |
| SEMA | Semaglutide Research Grade |
| BIO \| Anti-GLP-1 | Biotin Anti-GLP-1 |
| STRP \| HRP | Streptavidin:Horseradish Peroxidase |
| STRP \| HRP \| DIL | Streptavidin:HRP diluent |
| 1X \| CNTRL \| DIL | (1X) Control Diluent |
| 1X \| SAMP \| DIL | (1X) Sample Diluent |
| 20X \| WASH \| BUF | (20X) Wash Buffer |
| SUB \| TMB | TMB Substrate |
| SOLN \| STOP | Stop Solution |
|  | Consult Instructions for Use |
| REF | Catalog Number |
|  | Expiration Date |
|  | Storage Temperature |