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KRIBIOLISA® Anti-Semaglutide (Ozempic™) ELISA

REF : KBI9030

Ver 2.4

RUO

Enzyme Immunoassay for Quantitative Estimation of Antibodies to Semaglutide in human serum and plasma

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

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REF KBI9030 Σ 96 tests

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Introduction:

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 obtained in October 2017.

Patients can develop antibodies to Semaglutide due to its protein or peptide composition. The clinical significance of Anti-Semaglutide antibodies is generally low. In one clinical trial, only 2% of participants developed anti-drug antibodies, and these were all cross-reactions with endogenous GLP-1. Prolonged exposure to Semaglutide can lead to the development of neutralizing antidrug antibodies, which can reduce the safety and efficacy of the drug.

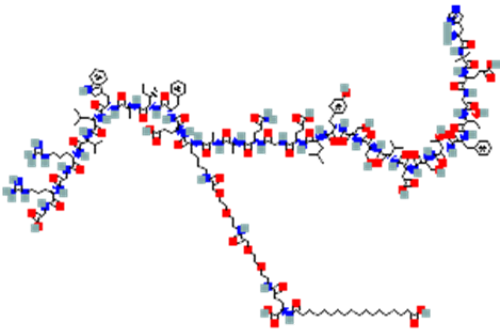
Trade Names:
OZEMPIC, Rybelsus, Wegovy

Generic Name:
Semaglutide

PubChem CID:
56843331

DrugBank Accession Number:
DB13928

Chemical Structure Depiction:

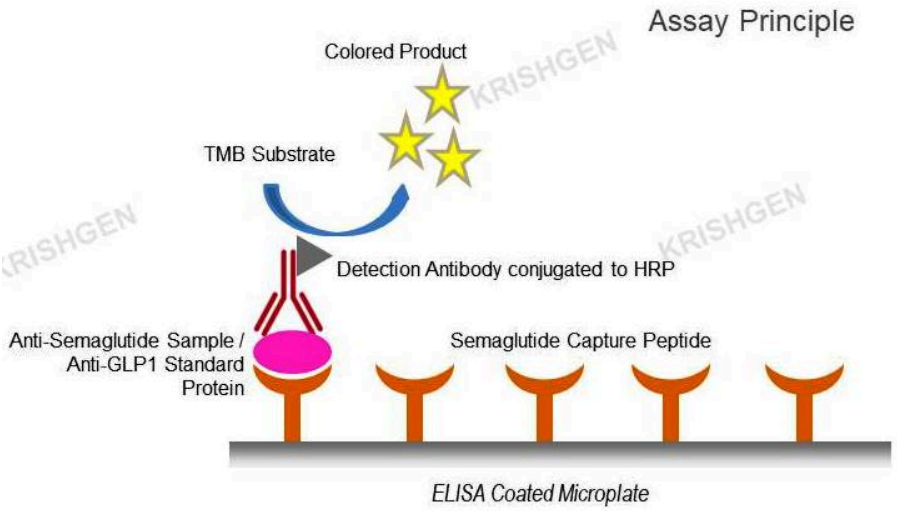


Intended Use:

The KRIBIOLISA® Anti-Semaglutide (Ozempic™) ELISA kit is used for estimation of antibodies to Semaglutide in human serum and plasma.

Principle:

The Anti-Semaglutide ELISA is a sandwich immunoassay for the determination of antibodies to Semaglutide. Semaglutide is coated on 96 well plates. Anti-GLP1 or antibodies to Semaglutide present in sample will bind to the plate. Plates are washed and Goat Anti-Mouse + Goat Anti-Human HRP conjugate is added which binds to bound antibodies. Washing is performed to remove any unbound material. TMB substrate is added and the enzyme reaction is stopped by dispensing of stop solution into the wells. The optical density (OD) of the solution at 450 nm is directly proportional to the amount of Anti –Semaglutide antibody present in standards or samples.



Materials Provided:

Part	Description	Qty
Semaglutide Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Semaglutide	1 x 96 wells
Anti-Semaglutide Standard	Recombinant Anti-Semaglutide standard Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane (lyophilized; 4000 ng/ml)	2 vials
Goat Anti-Mouse + Goat Anti-Human HRP conjugate Concentrated	Goat Anti-Mouse + Goat Anti-Human HRP conjugate Concentrated (1 mg/ml)	2 vials
Detection Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolinone and 0.02% bromonitrodioxane	12 ml
(1X) Standard Diluent	Buffered protein base with protein stabilizer and 1:1000 human serum and preservative 0.02% methylisothiazolinone and 0.02% bromonitrodioxane	10 ml
(1X) Sample Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolinone and 0.02% bromonitrodioxane	2 x 50 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersal <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. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.
9. Incubator.

Storage Information:

1. It is advisable to aliquot and store the Goat Anti-Mouse + Goat Anti-Human 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 Goat Anti-Mouse + Goat Anti-Human 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).
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

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 Serum & Plasma - Samples have to be diluted 1:1000 (v/v), e.g. 1 ul sample + 999 ul (1X) Sample Diluent prior to assay. The samples may be kept at 2-8°C for up to three days. Long-term storage requires -20°C.

Note:

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C
2. (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
3. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
4. When performing the assay, bring samples to room temperature.
5. It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

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 kit components and samples to room temperature (18-25°C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
3. To make Wash Buffer (1X); dilute 25 ml of (20X) Wash Buffer in 475 ml of DI water.
4. **Standards Preparation**: Reconstitute the concentrated Standard lyophilized vial with 500 ul of Standard diluent (1X) to obtain a concentration of 8000 ng/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Prepare further **Standards** by serially diluting the Top Standard (8000 ng/ml) as per the below table. Use the Standard Diluent (1X) as the Zero Standard (Standard No.0).

Standard Concentration	Standard No	Dilution Particulars
4000 ng/ml	Lyophilized Standard	Lyophilized Standard provided in the Kit
8000 ng/ml	Reconstituted standard	Lyophilized Standard provided in the Kit + 500 ul Standard Diluent (1X)
4000 ng/ml	Standard No.6	250 ul of Reconstituted standard + 250 ul of Standard Diluent (1X)
2000 ng/ml	Standard No.5	250 ul of Standard No. 6 + 250 ul Standard Diluent (1X)
1000 ng/ml	Standard No.4	250 ul of Standard No. 5 + 250 ul Standard Diluent (1X)
500 ng/ml	Standard No.3	250 ul of Standard No. 4 + 250 ul Standard Diluent (1X)
250 ng/ml	Standard No.2	250 ul of Standard No. 3 + 250 ul Standard Diluent (1X)
125 ng/ml	Standard No.1	250 ul of Standard No. 2 + 250 ul Standard Diluent (1X)
0 ng/ml	Standard No. 0	Only Standard Diluent (1X)

4. **Working Goat Anti-Mouse + Goat Anti-Human HRP conjugate – Refer to the Reagent Preparation sheet attached with the IFU and COA (enclosed in the kit).**

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 Anti-Semaglutide.
3. It is recommended that all 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 low / incorrect results.

6. The plates should be read within 30 minutes after adding the Stop Solution.

7. It is advisable to make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at 37°C

2. Pipette **100 ul** of prepared **Standards** or diluted **Samples** into the respective wells.

3. Cover the plate and incubate for 60 minutes at 37°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.

5. Pipette **100 ul** of **Working Goat Anti-Mouse + Goat Anti-Human HRP conjugate** into each well.

6. Cover the plate and incubate for 60 minutes at 37°C.

7. Aspirate and wash plate 4 times with **Wash Buffer (1X)** as mentioned in **Step 4**.

8. Add **100 ul** of **TMB Substrate** in each well.

9. Incubate the plate at 37°C for 30 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

10. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

11. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate Standards and Samples. Using Graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Anti-Semaglutide concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Anti-Semaglutide Concentration. If samples were diluted, multiply by the appropriate dilution factor.
Software which is able to generate a cubic spline or 4PL (2nd order) is best recommended for automated results.

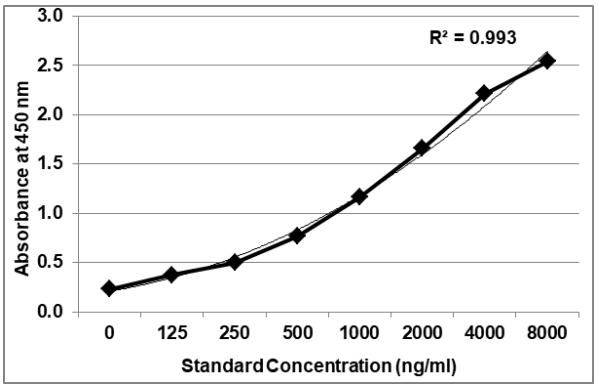
Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 8000 ng/ml standard.

Typical Data (representative only)

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 (representative only)



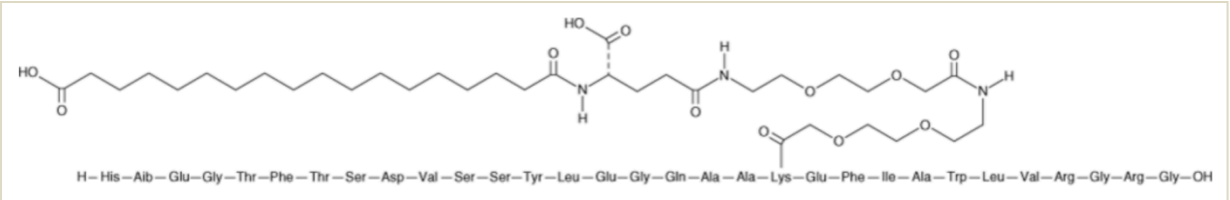
Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

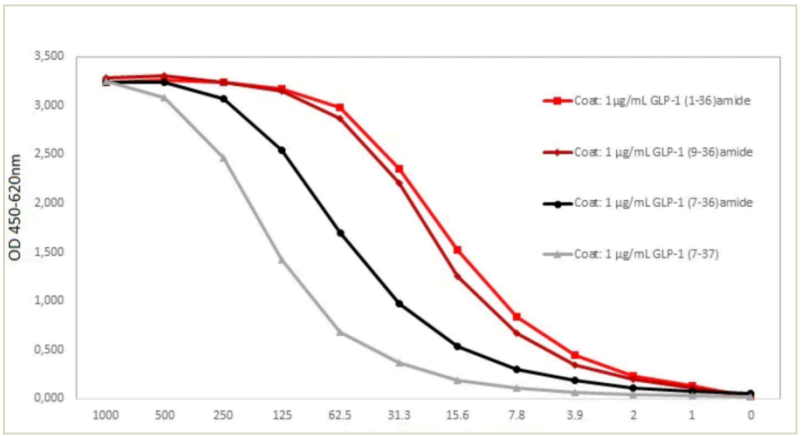
Performance Characteristics of the Kit:
This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

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.

Specificity:
The immobilized capture is a Semaglutide peptide which is synthetically prepared having a molecular formula C187H291N45O59 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% reactivity to Semaglutide. The Standard also cross reacts to other GLP-1 analogs and GLP-1s.



The ELISA graph displays different concentrations of the Standard used in the kit cross reacting with GLP-1 (1-36) amide, GLP-1 (9-36) amide, GLP-1 (7-36) amide, and GLP-1 (7-37).

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%

Lot to Lot Variation:

Standard Concentration (ng/ml)	Lot A OD450	Lot B OD450	Lot C OD450	Mean OD450	% STD DEV	CV	% CV
0	0.106	0.125	0.139	0.123	---	---	---
125	0.290	0.245	0.247	0.261	2.1	0.10	9.8
250	0.446	0.389	0.389	0.408	2.7	0.08	8.1
500	0.670	0.648	0.617	0.645	2.2	0.04	4.2
1000	0.979	1.058	1.154	1.064	7.2	0.08	8.3
2000	1.569	1.584	1.654	1.602	3.7	0.03	2.8
4000	2.126	2.064	1.925	2.038	8.4	0.05	5.0
8000	2.370	2.426	2.288	2.361	5.7	0.03	3.0

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/v) 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.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

Components (2-8ºC) → 30 mins RT Before Use → 

→ Thaw at Room Temperature (18-24ºC) → USE NOW

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID THAW (22±4ºC) → COOL (2-8ºC) → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

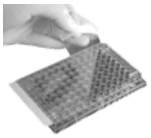
3.  Pipette **100 ul** prepared **Standards /** diluted **Samples** into each well.

4. Cover plate  and incubate for 

at 37°C.

5.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

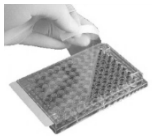
6.  Pipette **100 ul Working Goat Anti-Mouse + Goat Anti-Human HRP conjugate** into each well.

7 Cover plate  and incubate for 

at 37°C.

8.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

9.  Pipette **100 ul TMB Substrate** into each well.

10. Cover plate  and incubate for 

at 37°C.

11.  Pipette **100 ul Stop Solution** into each well.

12. Read absorbance at 450 nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml Anti -Semaglutide equivalent
1A 2A	0 ng/ml 0 ng/ml			
1B 2B	125 ng/ml 125 ng/ml			
1C 2C	250 ng/ml 250 ng/ml			
1D 2D	500 ng/ml 500 ng/ml			
1E 2E	1000 ng/ml 1000 ng/ml			
1F 2F	2000 ng/ml 2000 ng/ml			
1G 2G	4000 ng/ml 4000 ng/ml			
1H 2H	8000 ng/ml 8000 ng/ml			
3A 4A	Sample			
3B 4B	Sample			

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SYMBOLS KEY

MTP	aglutide Coated Microtiter Plate (12x8 wells)
STD	Semaglutide Standard, Lyophilized
HRP CONJ	ti-Mouse + Goat Anti-Human HRP conjugate rated
DETN DIL	on Diluent
1X STD DIL	dard Diluent
1X SAMP DIL	ple Diluent
20X WASH BUF	n Buffer
SUB TMB	ostrate
SOLN STOP	ution
	Consult Instructions for Use
REF	ogue Number
	Expiration Date
	Storage Temperature