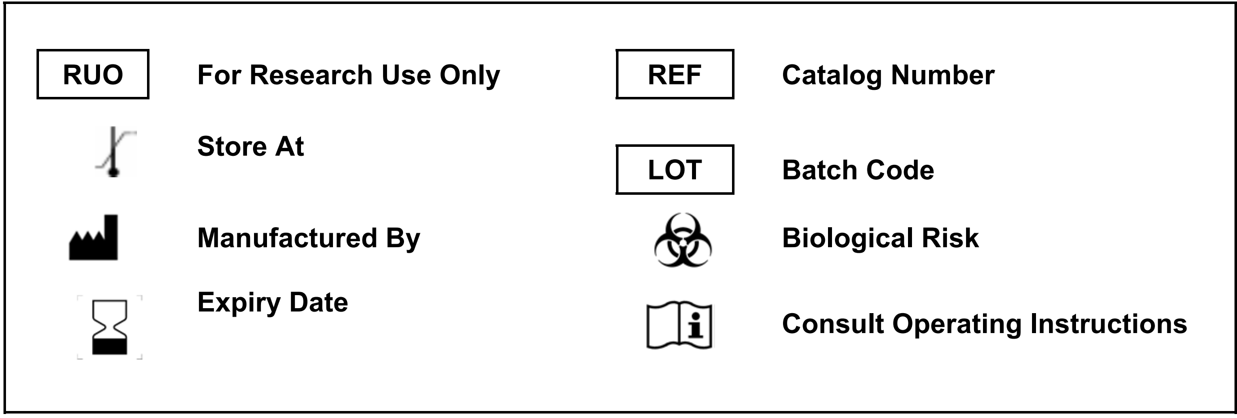
KRIBIOLISA™ Neutralizing Antibodies to Dulaglutide (TRULICITY) ELISA

REF : KBN1280

Ver 1.0

RUO

Enzyme Immunoassay for the Qualitative Detection of all types of Neutralizing Antibodies against Dulaglutide in serum or plasma.



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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.

Dulaglutide, marketed by Eli Lilly as Trulicity, is a once-weekly subcutaneous glucagon-like peptide-1 (GLP-1) receptor agonist designed using recombinant DNA technology; it has been approved as an adjunct therapy to diet and exercise in the management of 2 diabetes (T2DM).5 Dulaglutide was initially approved by the FDA in 2014, and in February 2020 was approved for use in patients with T2DM and multiple cardiovascular risk factors for the prevention of cardiovascular events. It is the first T2DM drug approved to reduce major adverse cardiovascular events (MACE) risk in primary and secondary prevention populations.

A neutralizing antibody (NAb) is an antibody that is responsible for defending cells from pathogens and are produced naturally by the body as part of its immune response. Their production is triggered by both infections and vaccinations against infections. In an immunogenetic context it will bind to a drug and neutralize its therapeutic effect.

Intended Use:

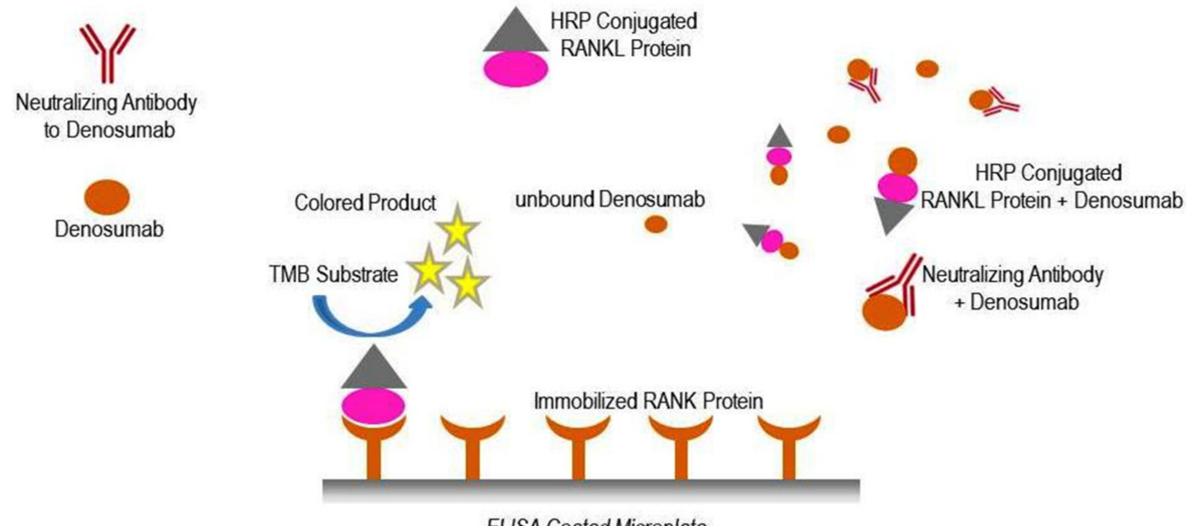
The KRIBIOLISA™ Neutralizing Antibodies to Dulaglutide (Trulicity) ELISA kit is used as an analytical tool for the qualitative detection of neutralizing antibodies against Dulaglutide in serum or plasma.

Principle:

The method employs sandwich ELISA technique. The protein-protein interaction between HRP-Dulaglutide and Anti-GLP1 can be blocked by Dulaglutide. The neutralizing antibodies against Dulaglutide binds to Dulaglutide and thus a competitive inhibition is created.

Samples and controls are pipetted in a blank microtitre plate and incubated with neutralizing antibody to Dulaglutide (NAb) and Dulaglutide. This complex of bound and unbound Dulaglutide is then incubated with HRP conjugated Dulaglutide protein. The unbound Dulaglutide will bind to the Anti-GLP1. The bound Nab-Dulaglutide will not bind to the HRP conjugated Dulaglutide.

This complex solution of bound antibodies to Dulaglutide and unbound Dulaglutide is then pipetted into Anti-GLP1 coated microplate. After washing, the substrate solution (TMB) is added to the microwells. Post incubation, color develops proportionate to the amount of bound Dulaglutide. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



PRINCIPLE OF THE KRIBIOLISA™ NEUTRALIZING ANTIBODIES TO DENOSUMAB ELISA

Materials Provided:

Part	Description	Qty
Anti-GLP1 Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-GLP1 protein	1 x 96 wells
Blank Microtiter Plate	96 well polystyrene uncoated microplate (12 strips of 8 wells)	1 x 96 wells
Neutralizing Anti-Dulaglutide Postive Control	Neutralizing Anti-Dulaglutide Positive Control (lyophilized)	2 vials
Neutralizing Anti-Dulaglutide Negative Control	Neutralizing Anti-Dulaglutide Negative Control (lyophilized)	2 vials
Dulaglutide	Dulaglutide Research Grade (lyophilized)	2 vials
Dulaglutide:HRP Conjugate	Dulaglutide:HRP Conjugate prepared in 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%	50 ml
(1X) Control Diluent	Buffered protein base with preservative thiomersol < 0.01% with 1:100 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 1:100 (v/v) for optimal recovery, (for example 1 ul sample + 99 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 Controls in 1ml Control Diluent.**
4. **Reconstitute the lyophilized Dulaglutide in 1ml Control Diluent.**
5. 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 Anti-Dulaglutide.
3. It is recommended that the Controls 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 Controls and Samples.

Assay Procedure:

1) Neutralization Reaction and Binding Reaction A

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.
4. Pipette **100 ul** of the prepared **Dulaglutide** into all the wells in the blank microplate.

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

6. Add **100 ul** of **Dulaglutide:HRP Conjugate** into all the wells.

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

2) Binding Reaction B

1. Pipette **100 ul** of the **Positive / Negative Control solution complex** into the respective wells of the Anti-GLP1 coated microplate from the neutralization reaction plate.

2. Pipette **100 ul** of the diluted **Samples solution complex** into the respective wells of the RANK coated microplate from the neutralization reaction plate.

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

4. Pipette **100 ul** of **TMB Substrate solution**.

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

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

7. **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	*0.261*	*0.284*	*0.584*

Therefore, Cut-Off = Mean + 2*SD
= 0.584 + (2*0.012)
= 0.584 + 0.024
= 0.608

Interpretation of Results:

Positive Samples *	**< Cut Off ***
Negative Samples *	**>= Cut Off ***

** The cutoff value is based on validation using recombinant antibodies in the assay. Users may set up their own cutoff values based on different patient serum panels from different geographic locations or ethnic backgrounds.*

Explanation od Results Interpetation:
i) If Neutralizing Antibody is present in the sample, it will bind to Dulaglutide. Dulaglutide will not be able to then bind to the Anti-GLP1. This in turn will lead to more binding of the Dulaglutide:HRP complex with Anti-GLP1 leading to a higher absorbance value.
ii) if Neutralizing Antibody is not present in the sample, it will lead to no binding (neutralizing) of Dulaglutide. Dulaglutide will then bind to the Anti-GLP1. This in turn will lead to less binding of the Dulaglutide:HRP complex with Anti-GLP1 leading to lower absorbance value.

Limitation of the Procedure:

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

Assay Range:
Qualitative

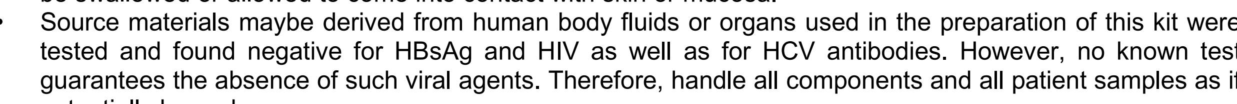
Precision:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Linearity:
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Human IL-6 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
serum (n=5)	84-107%	87-108%	82-112%
EDTA plasma (n=5)	83-102%	83-115%	83-118%
heparin plasma (n=5)	83-99%	80-95%	82-93%

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 2A	Negative Control Negative Control		
1B 2B	Postive Control Positive Control		
1C 2C	Sample Sample		
1D 2D	Sample Sample		
1E 2E	Sample Sample		
1F 2F	Sample Sample		
1G 2G	Sample Sample		
1H 2H	Sample Sample		
3A 4A	Sample Sample		
3B 4B	Sample Sample		
3C 4C	Sample Sample		

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SYMBOLS KEY

Symbol	Description
COATED MTP	Anti-GLP1 Coated Microtiter Plate (12x8 wells)
UNCOATED MTP	Uncoated Microtiter Plate (12x8 wells)
POS CTRL	Positive Control
NEG CTRL	Negative Control
CONJ HRP	Dulagluitde:Horseradish Peroxidase Conjugate
1X ASY DIL	(1X) Assay Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
i	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature