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KRIBIOLISA™ Neutralizing Antibodies to Imiglucerase (CEREZYME) ELISA

REF : KBN4030

Ver 1.0

RUO

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

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 KBN4030   ∑ **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.

Imiglucerase is a form of recombinant human beta-glucocerebrosidase enzyme used to replace the deficient endogenous enzyme in the treatment of Gaucher disease.

Human Beta-glucocerebrosidase or Beta-D-glucosyl-N-acylsphingosine glucohydrolase E.C. 3.2.1.45. 497 residue protein with N-linked carbohydrates, MW=59.3 kD. Alglucerase is prepared by modification of the oligosaccharide chains of human Beta-glucocerebrosidase. The modification alters the sugar residues at the non-reducing ends of the oligosaccharide chains of the glycoprotein so that they are predominantly terminated with mannose residues.

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 Imiglucerase (Cerezyme) ELISA kit is used as an analytical tool for the qualitative detection of neutralizing antibodies against Imiglucerase in serum or plasma.

Principle:

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

Samples and controls are pipetted in a blank microtitre plate and incubated with neutralizing antibody to Imiglucerase (NAb) and Imiglucerase. This complex of bound and unbound Imiglucerase is then incubated with HRP conjugated Imiglucerase protein.

This complex solution of bound antibodies to Imiglucerase and unbound Imiglucerase is then pipetted into Anti-Imiglucerase coated microplate. After washing, the substrate solution (TMB) is added to the microwells. Post incubation, color develops proportionate to the amount of bound HRP:Imiglucerase. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.
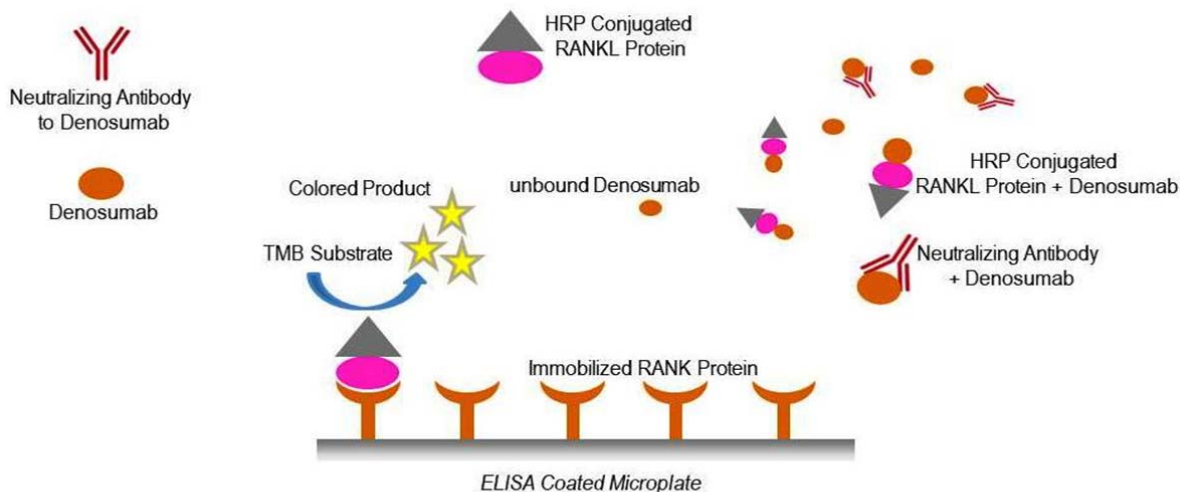
PRINCIPLE OF THE KRIBIOLISA™ NEUTRALIZING ANTIBODIES TO DENOSUMAB ELISA

Materials Provided:

Part	Description	Qty
Anti-Imiglucerase Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-Imiglucerase protein	1 x 96 wells
Blank Microtiter Plate	96 well polystyrene uncoated microplate (12 strips of 8 wells)	1 x 96 wells
Neutralizing Anti-Imiglucerase Postive Control	Neutralizing Anti-Imiglucerase Positive Control (lyophilized)	2 vials
Neutralizing Anti-Imiglucerase Negative Control	Neutralizing Anti-Imiglucerase Negative Control (lyophilized)	2 vials
Imiglucerase	Imiglucerase Research Grade (lyophilized)	2 vials
Imiglucerase:HRP Conjugate	Imiglucerase: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.

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

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.

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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.

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SYMBOLS KEY

COATED MTP	**Anti-Imiglucerase Coated Microtiter Plate (12x8 wells)**
UNCOATED MTP	**Uncoated Microtiter Plate (12x8 wells)**
POS CTRL	**Positive Control**
NEG CTRL	**Negative Control**
CONJ HRP	**Imiglucerase: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**