






KRIBIOLISA™ Obinutuzumab (Gazyva™) ELISA

REF : KBI1058

Ver 1.0


RUO

Enzyme Immunoassay for quantitative detection of Obinutuzumab (Gazyva™) in serum and plasma.

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

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 96 tests



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Introduction:

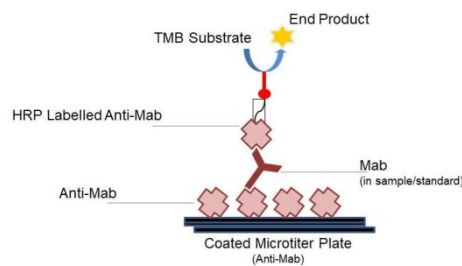
Obinutuzumab is a fully humanized monoclonal antibody that binds to an epitope on CD20 that partially overlaps with the epitope recognized by rituximab. Obinutuzumab binds to CD20 on B cells and causes these cells to be destroyed by engaging the adaptive immune system, directly activating intracellular apoptosis pathways, and activating the complement system.

Intended Use:

The KRIBIOLISA™ Obinutuzumab ELISA is used for quantitative detection of Obinutuzumab in serum and plasma.

Principle:

The method employs the quantitative sandwich enzyme immunoassay technique. Antibodies to Obinutuzumab are pre-coated onto microwells. Samples and standards are pipetted into microwells and human Obinutuzumab present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated Anti-Obinutuzumab is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Obinutuzumab in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



Materials Provided:

1. Anti-Obinutuzumab antibody Coated Microtiter Plate (12x8 wells) - 1 no
2. Obinutuzumab Standard (0.5 ml/vial) - 0, 50, 100, 200, 400, 800 and 1000 ng/ml
3. Anti-Obinutuzumab:HRP Conjugate - 12 ml
4. Assay Diluent - 50ml
5. (20X) Wash Buffer - 25 ml
6. TMB Substrate - 12 ml
7. Stop Solution - 12 ml
8. Instruction Manual

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. All reagents should be stored at 2⁰C to 8⁰C for stability.

2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25 °C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. 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.
2. For Research Use Only.

**Sample Preparation and Storage:**

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 Cell Culture Supernatant – If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Preparation Before Use:

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

Samples should be diluted 1:1000 (v/v), i.e. 1 ul sample + 999 ul sample diluent prior to assay. The samples may be kept at 2 - 8°C for up to three days. For long-term storage please store at -20°C.

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. To make Wash Buffer (1X); dilute 50 ml of 20X Wash Buffer in 950 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. High Dose Hook Effect may be observed in samples with very high concentrations of Obinutuzumab Antibody. High Dose Hook Effect is due to excess of very high concentrations of Obinutuzumab present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Obinutuzumab concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of Samples containing sodium azide (NaN₃), as it could destroy the HRP activity resulting in under-estimation of the amount of Obinutuzumab.
4. It is recommended that all Standards and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. 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.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. 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 to be run in duplicates. Pipette out **50 ul Assay Diluent** into the respective wells.
2. Pipette out **100 ul of Standard or Samples** into the respective wells as mentioned in the work list.
3. Cover the plate and incubate for 120 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 out **100 ul of Anti-Obinutuzumab:HRP Conjugate** in all wells.
6. Cover the plate and incubate for 60 minutes at 37°C.
7. Repeat the same procedure as in step 5 above.
8. Add **100 ul of TMB Substrate** in each well.
9. Incubate the plate at 37°C for 15-30 minutes at 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 reader

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Semi-Log graph paper, plot the average value (absorbance 450nm) 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 Obinutuzumab 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 Obinutuzumab Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit or using a polynomial regression to the 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 1000 ng/ml standard

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 50 ng/ml.

Specificity:

The antibodies used in the kit are anti-idiotypic, humanized monoclonal antibodies highly specific for Obinutuzumab. The standards are calibrated against commercially sourced Gazyva™.

Linearity:

Standards provided in the kit will be used for measuring the linearity range of Obinutuzumab present in matrix.

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 (200 ng/ml), medium (500 ng/ml) and high (800 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	<12%	<12%
High	<12%	<12%

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.



SCHEMATIC ASSAY PROCEDURE

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




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




3.  Pipette **25 ul Assay diluent** into the respective wells.

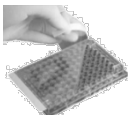

4. Pipette  **25 ul of Anti-Obinutuzumab:HRP Conjugate** into each well.

5.  Pipette **100 ul Standards / Samples** into the respective wells

6. Cover plate  and incubate for  at 37°C.

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

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

9. Cover plate  and incubate for  at 37°C in a Dark

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

11. Read absorbance at 450nm with a  microplate reader within  of stopping reaction.

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.



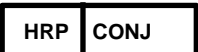









THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether 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.

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THANK YOU FOR USING KRISHGEN PRODUCT!

SYMBOLS KEY

	Anti-Obinutuzumab Microtiter Plate (12X8 wells)
	Obinutuzumab Standard
	Conjugate Horseradish Peroxidase
	Detection Antibody (20X)
	Assay Diluent
	Wash Buffer (20X)
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalogue Number
	Expiration Date
	Storage Temperature