

KRIBIOLISA™ Anti Omalizumab (XOLAIR) ELISA

REF : KBI2021

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Determination of Anti-Omalizumab in human serum and 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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 96 tests

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Introduction:

Omalizumab (trade name Xolair) is a fully humanized, engineered monoclonal antibody of IgG1 isotype against the protein programmed cell death-ligand 1 (PD-L1). In 2015, it was in clinical trials as an immunotherapy for several types of solid tumors. It was under investigation by Genentech/Roche. In April 2016, Roche announced that Omalizumab had been granted fast track status for lung cancer by the FDA. Anti-Drug Antibodies (ADA) may induce unwanted side effects in biopharmaceuticals. Hence, ADA has been subjected to increase in scrutiny by the regulatory authorities using immunogenicity safety studies. ADA has been observed in pre-clinical and clinical studies, resulting in significant changes in toxicology, pharmacokinetics and efficacy. These effects result from the generation of drug-induced (neutralizing) autoantibodies against Omalizumab and can be responsible for allergic reaction, or even anaphylactic shock.

Intended Use:

The KRIBIOLISA™ Anti-Omalizumab ELISA is used as an analytical tool for quantitative determination of Anti-Omalizumab in human serum and plasma.

Principle:

The method employs the sandwich immunoassay technique. Omalizumab is pre-coated onto microwells. Samples or standards are pipetted into microwells and antibodies to Omalizumab present in the sample are bound by the capture antibody. After washing microwells, HRP conjugate is pipetted and incubated. Free HRP conjugate will be removed by washing cycle. The ready to use substrate solution (TMB) is added to microwells and color develops directly proportionally to the amount of Anti-Omalizumab present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

Part	Description	Qty
Omalizumab Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Omalizumab monoclonal antibody.	1 x 96 wells
Anti-Omalizumab Standard	Lyophilized Anti-Omalizumab in a buffered protein base and preservative sodium azide < 0.01% - (lyophilized, concentrated 10 ug/ml)	2 vials
Omalizumab :HRP Conjugate	Omalizumab conjugated to Horseradish Peroxidase with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
(1X) Sample Diluent	Buffered protein base with preservative sodium azide < 0.01%	2 x 50 ml
(1X) Standard Diluent	Buffered protein base with preservative sodium azide < 0.01% with 1:1000 dilution of 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:

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1. All reagents should be stored at 2°C to 8°C 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.

Preparation Before Use:

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

Test Sample preparation - Samples have to be diluted 1:1000 (v/v), e.g. for 1:1000 (1 ul sample + 999 ul sample diluent) prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires the samples to be kept 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 25 ml of 20X Wash Buffer in 475 ml of DI water.
4. **Standards Preparation:** Reconstitute the concentrated Standard lyophilized vial with 500ul of Standard Diluent (1X) to obtain a concentration **20000 ng/ml Standard Solution**. Prepare further **Standards** by diluting the Standard Solution as per the below table. Use the Standard Diluent (1X) as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
10 ug/ml	Lyophilized Standard	Lyophilized Standard provided in the Kit + 500ul of Standard Diluent (1X)
20000 ng/ml	Standard No.7	Reconstituted Standard
10000 ng/ml	Standard No.6	250 ul Standard No.7 + 250 ul Standard Diluent (1X)
5000 ng/ml	Standard No.5	250 ul Standard No.6 + 250 ul Standard Diluent (1X)
2500 ng/ml	Standard No.4	250 ul Standard No.5 + 250ul Standard Diluent (1X)
1250 ng/ml	Standard No.3	250 ul Standard No.4 + 250 ul Standard Diluent (1X)
625 ng/ml	Standard No.2	250 ul Standard No.3 + 250 ul Standard Diluent (1X)
312.5 ng/ml	Standard No.1	250 ul Standard No.2 + 250ul Standard Diluent (1X)
0 ng/ml	Standard No.0	Only Standard Diluent (1X)

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 Anti-Omalizumab .
3. High Dose Hook Effect is due to excess of antibody for very high concentrations of Anti-Omalizumab 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

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compatible diluent. Thus if the Anti-Omalizumab concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.

4. 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-Omalizumab .
5. It is recommended that all Standards and Samples be assayed in duplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. 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.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. 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. Add **100 ul** of **Standards** or **Samples** into the respective wells.
3. Cover the plate and incubate for 180 minutes at 37°C.
4. Aspirate and wash plate 4 times with no soak time 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. Add **100 ul** of **Omalizumab :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 no soak time 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.
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 reader.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Standard 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 Anti- Omalizumab 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-Omalizumab Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4PL is best recommended for automated results.

Note:

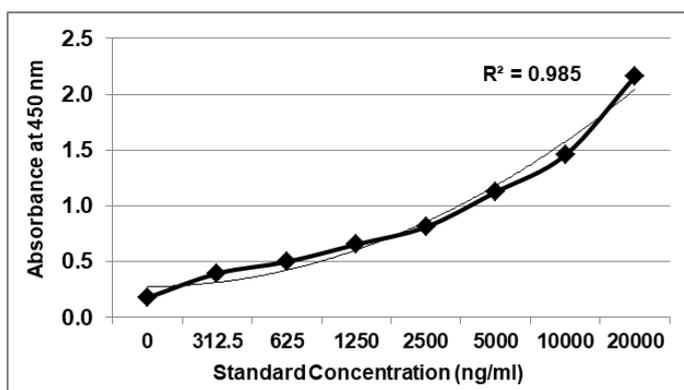
It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 20000 ng/ml standard.

Typical Data

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.181	0.176	0.179		--
312.5	0.393	0.399	0.396	308.0	98.6
625	0.505	0.495	0.500	665.4	106.5
1250	0.652	0.660	0.656	1425.3	114.0
2500	0.818	0.808	0.813	2441.4	97.7
5000	1.136	1.116	1.126	5196.2	103.9
10000	1.454	1.460	1.457	9103.9	91.0
20000	2.161	2.159	2.160	20582.5	102.9

Typical Graph



abs= absorbance @450nm

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 310 ng/ml.

Limit Of Quantification:

It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 312.5 ng/ml.

Specificity:

The antibodies used in the kit are monoclonal antibodies, anti-idiotypic and specific for Anti-Omalizumab.

Linearity:

Standards provided in the kit will be used for measuring the linearity range of Anti-Omalizumab 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 (312.5ng/ml), medium (2500ng/ml) and high (20000ng/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%

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.

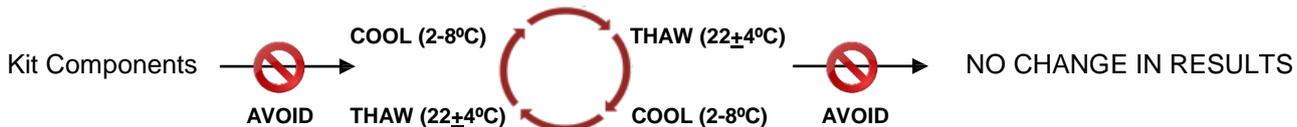


SCHEMATIC ASSAY PROCEDURE

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

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

5. Aspirate and wash wells 4 times with **Wash Buffer (1X) (no soak time)**.

6. Pipette **100 ul Omalizumab :HRP** into each well.

7. Cover plate and incubate for at 37°C

8. Aspirate and wash wells 4 times with **Wash Buffer (1X). (no soak time)**.

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 450nm with a microplate reader within of stopping reaction.

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SYMBOLS KEY

	Omalizumab Coated Microtiter Plate (12X8 wells)
	Anti-Omalizumab Standard, lyophilized
	Conjugate Horseradish Peroxidase
	(1X) Sample Diluent
	(1X) Standard Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalogue Number
	Expiration Date
	Storage Temperature