KRIBIOLISA™ Benralizumab (FASENRA) ELISA

REF : KBI1183

Ver 1.2

RUO

Enzyme Immunoassay for the quantitative determination of Benralizumab in human serum and plasma.

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biologi isk
	Expiry D		Consu rating Instructions

For Research Use Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.

REF KBI1183 Σ 96 tests

KRISHGEN BioSystems
For US / Europe: toll free +1(888)-970-0827 tel: +1(562)-568-5005,
For Asia / India: tel: +91(22)-49198700.
Email: sales1@krishgen.com

Introduction:

Benralizumab is a monoclonal antibody is used for the treatment of asthma. It is directed against the alpha-chain of the interleukin-5 receptor (CD125). The humanized monoclonal antibody benralizumab specifically binds to IL-5Rα, thereby preventing the interaction between IL-5 and its receptor. In addition, through its Fc constant region, benralizumab binds to the FcγIIIRa receptor expressed by natural killer cells, thus inducing eosinophil apoptosis operated by the release of proapoptotic proteins such as granzymes and perforins.

Intended Use:

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

Principle:

The method employs the quantitative sandwich enzyme immunoassay technique. Human IL5RA/CD125 Protein pre-coated onto microwells. Samples and standards are pipetted into microwells and human Benralizumab present in the sample are bound by the capture protein. Then, a HRP (horseradish peroxidase) conjugated anti-human IgG antibody 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 Benralizumab in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

Part	Description	Qty
Human IL5RA/CD125 Protein Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Human IL5RA/CD125 Protein.	1 x 96 wells
Benralizumab Standard, (lyophilized)	Recombinant Benralizumab in a buffered protein base and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane – (lyophilized, concentrated 4 ug/ml)	2 vials
Anti-Human IgG:HRP Conjugate Concentrated	Anti-human IgG conjugated to Horseradish Peroxidase concentrated (1 mg/ml)	1 vial
(1X) Standard Diluent	Buffered protein base with preservative 0.02% methylisothiazolone and 0.02% bromonitrodioxane and 1:1000 dilution human serum	10 ml
Detection Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
(1X) Sample Diluent	Buffered protein base with preservative 0.02% methylisothiazolone and 0.02% bromonitrodioxane	2 x 50 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. All reagents should be stored at 2ºC to 8ºC 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 (18-25ºC). 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 -80ºC. 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.

Test Sample preparation:

- Serum 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 -20°C.
- Plasma 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 -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 lyophilized standard in 1000ul of Standard diluent to get a concentration of 4000 ng/ml. Keep the standard for 15 minutes. Prepare the remaining standards as per the below table. Standard Diluent (1X) serves as the zero standard (0 ng/ml)

Standard Concentration nn	Standard Vial	Dilution Particulars
4 ug/ml	Lyophilized Standard	Lyophilized Std. provided in the Kit + 1ml of Standard Diluent (1X)
4000 ng/ml	Standard No.7	Reconstituted Standard
2000 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Standard Diluent (1X)
1000 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent (1X)
500 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent (1X)
250 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent (1X)
125 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent (1X)
62.5 ng/ml	Standard No. 1	500 ul Standard No.2 + 500 ul Standard Diluent (1X)
0 ng/ml	Standard No.0	Only Standard Diluent (1X)

Use the prepared Standards as soon as possible upon reconstitution. Discard balance prepared standards after use.

5. **Working Anti-Human IgG: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. High Dose Hook Effect may be observed in samples with very high concentrations of Benralizumab. High Dose Hook Effect is due to excess of antibody for very high concentrations of Benralizumab 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 Benralizumab 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 (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Benralizumab.
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 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 **Standards** or **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. Add **100 ul** of **Working Anti-Human IgG :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)** 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 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 Benralizumab 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 Benralizumab Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit 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 4000 ng/ml standard.

Quality Control:

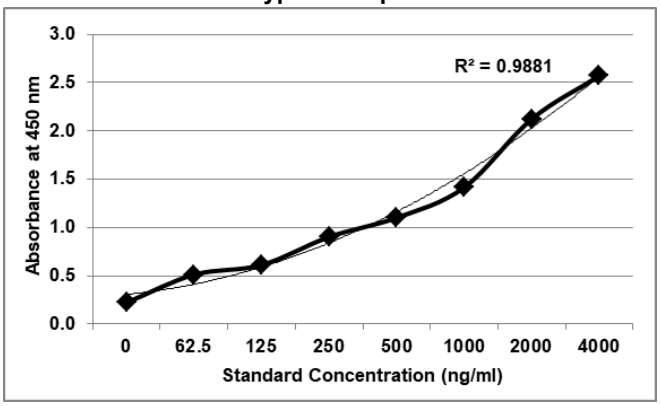
It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Typical Data

(for reference only, please refer to the CoA for lot specific data)

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.224		--
62.5	0.513	69.1	110.6
125	0.613	114.1	91.3
250	0.904	299.4	119.7
500	1.102	474.9	95.0
1000	1.416	848.8	84.9
2000	2.124	2288.3	114.4
4000	2.569	3860.5	96.5

Typical Graph



Abs = absorbance at 450nm

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.

Specificity:

The standard / calibrator antibodies used in the kit are monoclonal antibodies, anti-idiotypic and specific for Benralizumab.

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 60 ng/ml

Linearity:
Standards provided in the kit will be used for measuring the linearity range of Benralizumab 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 (62.5ng/ml), medium (500ng/ml) and high (4000ng/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.

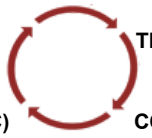
SCHEMATIC ASSAY PROCEDURE

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

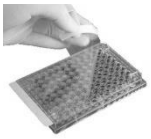
Components (2-8ºC) → 30 mins RT Before Use → 

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

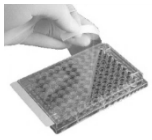
3.  Pipette **100 ul Standards** / **Samples** into each well.

4. Cover plate  and incubate for 

at 37°C.

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

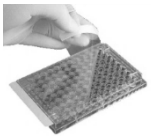
6.  Pipette **100 ul Working Anti human IgG:HRP** into each well.

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 450nm with a  microplate reader within  of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml Benralizumab equivalent
1A 2A	zero std zero std			
1B 2B	62.5 ng/ml 62.5 ng/ml			
1C 2C	125 ng/ml 125 ng/ml			
1D 2D	250 ng/ml 250 ng/ml			
1E 2E	500 ng/ml 500 ng/ml			
1F 2F	1000 ng/ml 1000 ng/ml			
1G 2G	2000 ng/ml 2000 ng/ml			
1H 2H	4000 ng/ml 4000 ng/ml			
3A 4A	Sample			
3B 4B	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.

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.

Krishgen
2024

THANK YOU KRISHGEN

Biosystems.

FOR USING PRODUCT!

SYMBOLS

KEY

	H L5RA/CD125 Protein Coated M MTP Plate (12x8 wells)
	B STD mab Standard (lyophilized)
	HRP CONJ gG:HRP Conjugate concentrated
	DETN DIL iluent
	20X WASH BUF uffer
	1X SAMP DIL Diluent
	1X STD DIL d Diluent
	SUB TMB te
	SOLN STOP n
	Consult Instructions for Use
	C REF umber
	Expiration Date
	Storage Temperature