






# KRIBIOLISA® Anti-Ranibizumab (Lucentis™) ELISA

**REF** : KBI2029

Ver 1.1


**RUO**

Enzyme Immunoassay for the Quantitative Determination of Anti-Ranibizumab in human serum and plasma

<b>RUO</b>	For Research Use Only	<b>REF</b>	Catalog Number
	Store At	<b>LOT</b>	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating 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 Private Limited is strictly prohibited.*

**REF** KBI2029

 96 tests

**Krishgen Biosystems Private Limited**

For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005

For Asia/India Customers: tel +91(22)-49198700

Email: sales1@krishgen.com | <http://www.krishgen.biz> / [www.krishgenbio.com](http://www.krishgenbio.com)

**Introduction:**

Ranibizumab (Lucentis™) is a recombinant humanized IgG1 monoclonal antibody fragment that binds to and inhibits vascular endothelial growth factor A (VEGF-A). VEGF is a biochemical signal protein that promotes angiogenesis throughout the body and in the eye.

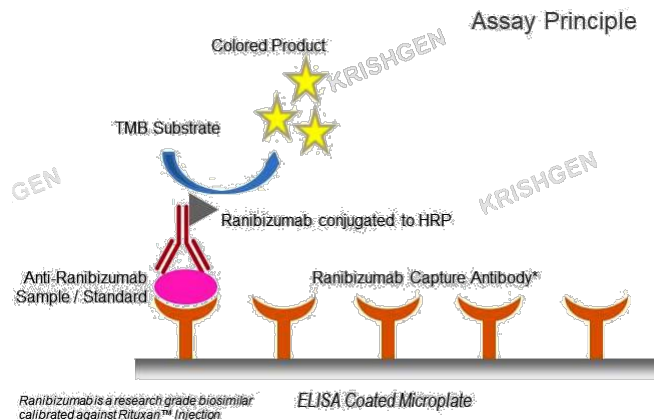
Anti-Drug Antibodies (ADA) may induce unwanted side effects in biopharmaceuticals. Hence, ADA has been subjected to increase in scrutiny by the regulatory authorities using immunogenicity safety studies. ADA has been observed in pre-clinical and clinical studies, resulting in significant changes in toxicology, pharmacokinetics and efficacy. These effects result from the generation of drug-induced (neutralizing) autoantibodies against Ranibizumab and can be responsible for allergic reaction, or even anaphylactic shock. This ELISA kit detects antibodies for Anti-Ranibizumab and may be used for monitoring immunogenicity

**Intended Use:**

The KRIBIOLISA® Anti-Ranibizumab (LUCENTIS™) ELISA is used as an analytical tool for quantitative determination of Anti-Ranibizumab in human serum and plasma.

**Principle:**

The method employs the sandwich enzyme immunoassay technique. Ranibizumab is pre-coated onto microwells. Samples or standards are pipetted into microwells and antibodies to Ranibizumab present in the standards or sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated Ranibizumab is pipetted and incubated. After washing microwells in order to remove any nonspecific binding, the ready to use 3, 3', 5, 5' Tetra Methyl Benzidine (TMB) substrate solution is added to microwells and color develops proportionally to the amount of Anti-Ranibizumab in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



**Materials Provided:**

Part	Description	Qty
Ranibizumab Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Recombinant Ranibizumab	1 x 96 wells
Anti- Ranibizumab Standard	Recombinant Anti-Ranibizumab Standard in a buffered protein base with protein stabilizer and preservatives 0.02% Methylisothiazolinone and 0.02% bromonitrodioxane (lyophilized – 1 ug/ml)	2 vials
Ranibizumab:HRP Conjugate concentrated	Ranibizumab:HRP Conjugate concentrated (1 mg/ml)	2 vials
Detection Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolinone and 0.02% bromonitrodioxane	12 ml
(1X) Sample Diluent	Buffered protein base with protein stabilizer and preservatives 0.02%, methylisothiazolinone and 0.02% bromonitrodioxane	2 x 50 ml
(1X) Standard Diluent	Buffered protein base with 1:1000 dilution human serum and preservatives 0.02% Methylisothiazolinone and 0.02% bromonitrodioxane	10 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersal < 0.01%. May turn yellow over time	25 ml

Part	Description	Qty
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. It is advisable to aliquot and store the Ranibizumab:HRP Conjugate concentrated at -20°C upon receipt. Rest of the kit components should be stored at 2-8°C. Immediately discard any excess Working Ranibizumab:HRP Conjugate after running your assay.
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 - Serum and 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 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 500 ul of Standard Diluent (1X) to obtain a concentration of 2000 ng/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. 2000 ng/ml is the top standard. Prepare further **Standards** by serially 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
1 ug/ml	Lyophilized Standard	Lyophilized Standard provided in the Kit
2000 ng/ml	Standard No.7	Reconstitute in 500 ul of Standard Diluent (1X)
1000 ng/ml	Standard No.6	250 ul Standard No.7 + 250 ul Standard Diluent (1X)
500 ng/ml	Standard No.5	250 ul Standard No.6 + 250 ul Standard Diluent (1X)
250 ng/ml	Standard No.4	250 ul Standard No.5 + 250 ul Standard Diluent (1X)
125 ng/ml	Standard No.3	250 ul Standard No.4 + 250 ul Standard Diluent (1X)
62.5 ng/ml	Standard No.2	250 ul Standard No.3 + 250 ul Standard Diluent (1X)
31.25 ng/ml	Standard No.1	250 ul Standard No.2 + 250 ul Standard Diluent (1X)
0 ng/ml	Standard No.0	Only Standard Diluent (1X)

Use the Standards immediately upon reconstitution. Discard balance standard after use. Do not store them for further experiments.

**5. Working Ranibizumab: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 Anti-Ranibizumab. High Dose Hook Effect is due to excess of antibody for very high concentrations of Anti-Ranibizumab present in the sample.
3. Avoid assay of Samples containing sodium azide ( $\text{NaN}_3$ ), as it could destroy the HRP activity resulting in under-estimation of the amount of Anti-Ranibizumab.
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. All steps must be performed at 37°C.
2. Pipette **100 ul** of **prepared Standards and diluted Samples** to the respective wells.
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 **100 ul** of **working Ranibizumab:HRP Conjugate** to all wells.
6. Cover the plate and incubate for 60 minutes at 37°C.
7. Aspirate and wash plate 4 times with **Wash Buffer (1X)** as mentioned in Step 4.
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 within 10-15 minutes after addition of Stop solution.

### Interpretation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using 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-Ranibizumab 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-Ranibizumab Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit or a 4PL polynomial curve (2<sup>nd</sup> order) is best recommended for automated results.

### Note:

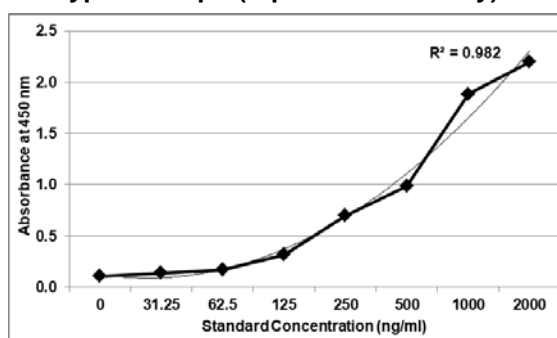
It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 2000 ng/ml standard.

### Typical Data (representative only)

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.107	--	--
31.25	0.140	28.4	91.0
62.5	0.171	51.2	82.0
125	0.319	128.7	103.0
250	0.699	292.3	116.9
500	0.986	423.8	84.8
1000	1.885	1146.2	114.6
2000	2.202	1850.7	92.5

### Typical Graph (representative only)



### 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 Quantification:** It is defined as the lowest concentration of an analyte that can be determined with an acceptable repeatability and the LOQ was found to be 30 ng/ml.

KRIBIOLISA® Anti-Ranibizumab (LUCENTIS™) ELISA

**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 30 ng/ml

**Specificity:**

The capture antibody is a biosimilar research grade Ranibizumab calibrated with commercially sourced Lucentis™ Injection. The standards used in the kit are proteins are recombinantly expressed Ranibizumab used for capture and detection.

The calibrator / standard used is a human Anti-Ranibizumab antibody. The calibrator / standard is a paratope specific, inhibitory anti-idiotypic antibody in IgG1 format that recognizes the monoclonal antibody drug Ranibizumab. The antibody does not recognize recombinant human vascular endothelial growth factor A (VEGF-A) or ranibizumab when in complex with recombinant human VEGF-A. This antibody also recognizes the Bevacizumab.

**Spike and Recovery**

A known amount of analyte (Anti-Ranibizumab) was added (spiked) to normal human serum. The assay was run to measure the response (recovery) of the spiked sample matrix compared to an identical spike in the standard diluent.

Sample Type	No Spike (0 ng/ml)	Low Spike (31.25 ng/ml)	Medium Spike (250 ng/ml)	High Spike (2000 ng/ml)
Standard Diluent	0.00	31.20	254.00	2090.10
Serum Sample 1	0.70	30.80	253.40	2020.12
Serum Sample 2	0.00	32.09	252.19	1995.25
Serum Sample 3	0.60	31.00	250.95	1987.01
% Mean Recovery	NA	100.1%	101.10%	101.20%

**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 (31.25 ng/ml), medium (500 ng/ml) and high (2000 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	<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/v) 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



KRIBIOLISA® Anti-Ranibizumab (LUCENTIS™) ELISA

**References:**

Anti-drug antibodies to brolicizumab and ranibizumab in serum and vitreous of patients with ocular disease  
M Busch, JM Pfeil, M Dähmcke... - Acta ..., 2022 - Wiley Online Library

Development and Implementation of an ELISA to Detect "Anti-Ranibizumab" Immunity in Age-Related Macular Degeneration Patients  
A Magno, M Lai, C Pierce, K Davern... - ... & Visual Science, 2013 - iovs.arvojournals.org

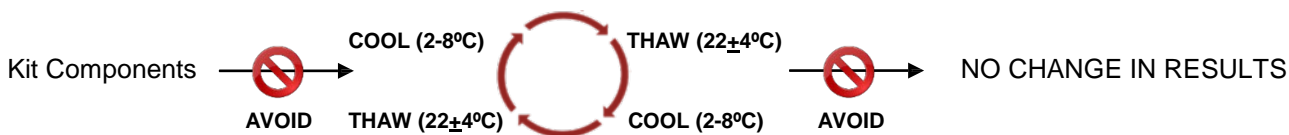
Preclinical pharmacokinetics of Ranibizumab (rhuFabV2) after a single intravitreal administration  
J Gaudreault, D Fei, J Rusit, P Suboc... - ... ophthalmology & visual ..., 2005 - iovs.arvojournals.org

**SCHEMATIC ASSAY PROCEDURE**

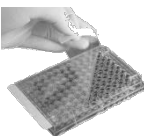

1. Remove all components, 30 minutes before adding into the assay plate.




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

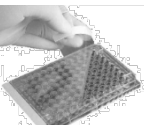




3.  Pipette **100 ul prepared Standards / diluted Samples** to the respective wells.

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

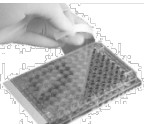

5.  Aspirate and wash wells 4 times with **Wash Buffer (1X)**.

6.  Pipette **100 ul working Ranibizumab:HRP conjugate** 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 450 nm with a  microplate reader within  of stopping reaction.

## LIMITED WARRANTY

Krishgen Biosystems Private Limited 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 Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited 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 Private Limited.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems Private Limited shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems Private Limited 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 Private Limited be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited 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 Biosystems Private Limited. 2025

## THANK YOU FOR USING KRISHGEN PRODUCT!




KRISHGEN BIOSYSTEMS PRIVATE LIMITED®, GENLISA®, DHARMAPLEX®, GENBULK®, GENLISA®, KRISHZYME®, KRISHGEN®, KRIBIOLISA®, KRISHPLEX®, TITANIUM®, QUALICHEK® are registered trademarks of KRISHGEN BIOSYSTEMS PRIVATE LIMITED.

©KRISHGEN BIOSYSTEMS PRIVATE LIMITED. ALL RIGHTS RESERVED.

KRISHGEN BIOSYSTEMS PRIVATE LIMITED | OUR REAGENTS | YOUR RESEARCH |

**LUCENTIS is the registered trademark of Genentech Inc.,**

### SYMBOLS KEY

<b>MTP</b>	Ranibizumab Coated Microtiter Plate (12x8 wells)
<b>STD</b>	Anti-Ranibizumab Standard, Lyophilized
<b>HRP CONJ</b>	Ranibizumab:HRP Conjugate concentrated
<b>DETN DIL</b>	Detection Diluent
<b>1X SAMP DIL</b>	(1X) Sample Diluent
<b>1X STD DIL</b>	(1X) Standard Diluent
<b>20X WASH BUF</b>	(20X) Wash Buffer
<b>SUB TMB</b>	TMB Substrate
<b>SOLN STOP</b>	Stop Solution
	Consult Instructions for Use
<b>REF</b>	Catalog Number
	Expiration Date
	Storage Temperature