

KRIBIOLISA® Anti-Vedolizumab (ENTYVIO™) ELISA

REF : KBI2096

Ver 1.1

RUO

Enzyme Immunoassay for the Quantitative Determination of antibodies to Vedolizumab (ENTYVIO™) in serum, plasma and cell culture supernatant

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:

Anti-vedolizumab antibodies are anti-drug antibodies (ADAs) generated by the immune system against vedolizumab, a recombinant humanized IgG1 monoclonal antibody that selectively targets the $\alpha 4\beta 7$ integrin. These antibodies may develop during vedolizumab treatment and can bind to the therapeutic antibody, potentially interfering with its ability to bind to $\alpha 4\beta 7$ integrin and exert its pharmacological effect. The development of anti-vedolizumab antibodies may therefore result in increased drug clearance, reduced vedolizumab exposure, and, in some patients, a diminished or lost therapeutic response.

Intended Use:

The KRIBIOLISA® Anti-Vedolizumab (ENTYVIO™) ELISA is used as an analytical tool for quantitative determination of antibodies to Vedolizumab (ENTYVIO™) in serum, plasma and cell culture supernatant.

Principle:

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

Materials Provided:

Part	Description	Qty
Vedolizumab Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Vedolizumab.	12 x 8 wells
Anti- Vedolizumab Standard	Recombinant Anti-Vedolizumab standard – (lyophilized – 2 ug/ml)	2 vials
Vedolizumab:HRP conjugate, concentrated	Vedolizumab conjugated to HRP (concentrated, 1 mg/ml – 8 ul)	2 vials
Detection Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% Methylisothiazolinone and 0.02% bromonitrodioxane.	12 ml
(1X) Standard Diluent	Buffered protein base with protein stabilizer and 1:100 human serum and preservative thiomersol < 0.01%	10 ml
(1X) Sample Diluent	Buffered protein base with BSA and preservative thiomersol < 0.01%.	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 25µl to 1000µl
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 Vedolizumab: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 Vedolizumab:HRP Conjugate, concentrated 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.

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.

Test Sample preparation –

- Serum Samples have to be diluted 1:100 (v/v), e.g. for 1:100 (1 ul sample + 99 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. **Standard Preparation:** Reconstitute the lyophilized standard in 1 ml of (1X) Standard diluent to get a concentration of 2000 ng/ml. Keep the standard for 15 minutes. 2000 ng/ml is the top standard. Prepare the remaining standards as per the below table. Standard Diluent (1X) serves as the zero standard (0 ng/ml)

Standard Concentration	Standard No	Dilution Particulars
2000 ng/ml	Standard No. 7	Lyophilized Standard provided in the Kit + 1000ul of Standard Diluent (1X).
1000 ng/ml	Standard No. 6	500ul of Standard No. 7 + 500 ul of Standard Diluent (1X)
500 ng/ml	Standard No.5	500 ul of Standard No. 6 + 500 ul Standard Diluent (1X)
250 ng/ml	Standard No.4	500 ul of Standard No. 5 + 500 ul Standard Diluent (1X)
125 ng/ml	Standard No.3	500 ul of Standard No. 4 + 500 ul Standard Diluent (1X)
62.5 ng/ml	Standard No.2	200 ul of Standard No. 3 + 800 ul Standard Diluent (1X)
31.25 ng/ml	Standard No.1	500 ul of Standard No. 2 + 500 ul Standard Diluent (1X)
0 ng/ml	Standard No. 0	Only Standard Diluent (1X)

5. **Working Vedolizumab: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-Vedolizumab (ENTYVIO™). High Dose Hook Effect is due to excess of antibody for very high concentrations of Anti-Vedolizumab (ENTYVIO™) 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 Anti-Vedolizumab (ENTYVIO™) 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 Anti-Vedolizumab (ENTYVIO™).
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 compromise 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 Controls and Samples be run in duplicates or triplicates. A standard curve is required for each assay.
2. Pipette **100 ul** of **Standards** or **Samples** into the respective wells.
3. Cover the plate and incubate for 60 minutes at Room Temperature (18-25°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 Vedolizumab:HRP Conjugate** into each well.
6. Cover the plate and incubate for 60 minutes at Room Temperature (18-25°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 Room Temperature 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.

Interpretation 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 Anti-Vedolizumab 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-Vedolizumab 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:

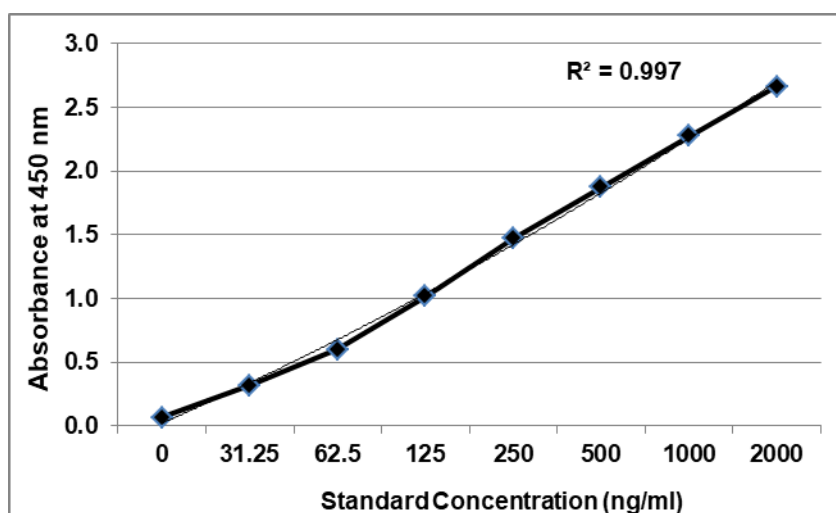
It is recommended to repeat the assay at a different dilution factor in the following cases:

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

Typical Data (representative only)

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.061	0.077	0.069	1.8	--
31.25	0.304	0.335	0.320	25.3	80.9
62.5	0.616	0.586	0.601	60.3	96.5
125	1.004	1.033	1.018	134.3	107.5
250	1.449	1.495	1.472	265.6	106.2
500	1.874	1.872	1.873	473.6	94.7
1000	2.301	2.255	2.278	917.9	91.8
2000	2.725	2.601	2.663	2238.9	111.9

Typical Graph

**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.

Standard Calibration Range:

31.25 ng/ml – 2000 ng/ml.

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 28 ng/ml

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 (31.25ng/ml), medium (250ng/ml) and high (2000ng/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%

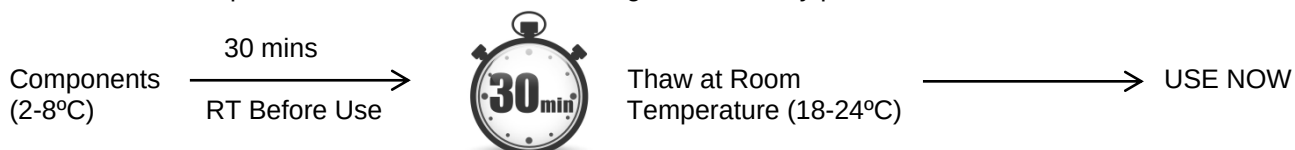
Safety Precautions:

- **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.
- 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 µl prepared Standards / diluted Samples** into each well.

4. Cover plate and incubate for at Room Temperature.

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

6. Pipette **100 µl Working Vedolizumab:HRP Conjugate** into each well.

7. Cover plate and incubate for at Room Temperature.

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

9. Pipette **100 µl TMB Substrate** into each well.

10. Cover plate and incubate for at Room Temperature.

11. Pipette **100 µl 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 Anti Vedolizumab (ENTYVIO™) equivalent
1A 2A	zero std zero std			
1B 2B	31.25 ng/ml 31.25 ng/ml			
1C 2C	62.5 ng/ml 62.5 ng/ml			
1D 2D	125 ng/ml 125 ng/ml			
1E 2E	250 ng/ml 250 ng/ml			
1F 2F	500 ng/ml 500 ng/ml			
1G 2G	1000 ng/ml 1000 ng/ml			
1H 2H	2000 ng/ml 2000 ng/ml			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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SYMBOLS KEY

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