MATRIKS BIOTEK

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Instructions for Use

SHIKARI® (S-ATRAV)

Quantitative Antibodies to Ravulizumab ELISA

Enzyme immunoassay for determination of quantitative antibodies to Ravulizumab in serum and plasma samples

REF	**RAV-QNS-ULT**
Σ	96 tests
	Shipment 10-30°C, Store 2-8°C
	MATRIKS BIOTECHNOLOGY CO., LTD. Bahcelievler Mah. 323/1 Cad. Gazi Universitesi Teknokent Binası C Blok No:10/50C/47 06830 Golbasi Ankara / TURKEY Tel +90 312 485 42 94 info@matriksbiotek.com
CE IVD	

1. Intended Use

SHIKARI® Quantitative Antibodies to Ravulizumab ELISA has been especially developed for the quantitative analysis of antibodies to Ravulizumab in serum and plasma samples. SHIKARI® Quantitative Antibodies to Ravulizumab ELISA is optimized with Ultomiris®.

2. General Information

Ravulizumab is a potent and selective complement 5 (C5) inhibitor. It is a humanized monoclonal IgG2/4 kappa antibody produced in Chinese hamster ovary (CHO) cells. Ravulizumab was engineered from eculizumab, another complement inhibitor, to increase the duration of action and reduce the frequency of drug administration. It works by blocking terminal complement-mediated inflammation, cell activation, and cell lysis in blood disorders associated with the destruction of red blood cells, thrombosis, and impaired bone marrow function. Paroxysmal nocturnal hemoglobinuria (PNH) is a hematopoietic stem cell disorder characterized by hemolytic anemia, bone marrow failure, and thrombosis. It is caused by a genetic mutation, leading to complement-mediated hemolysis and deficiencies in glycosylphosphatidylinositol (GPI)-linked proteins such as those involved in fibrinolysis. Ravulizumab inhibits the terminal complement pathway by binding to C5 with high affinity: this inhibits the cleavage of C5 to C5a, which is a pro-inflammatory and pro-thrombotic anaphylatoxin, and C5b, an initiating subunit of the terminal complement complex (C5b-9), which promotes cell lysis. Since the generation of C5b is blocked, the formation of C5b-9 is also inhibited by ravulizumab. Ravulizumab inhibits terminal complement-mediated intravascular hemolysis in patients with PNH and complement-mediated thrombotic microangiopathy (TMA) in patients with aHUS. By blocking the complement system, ravulizumab ameliorates the extent of inflammatory and immune responses that play a role in the pathophysiology of myasthenia gravis.

Therapeutic drug monitoring (TDM) is the clinical practice of measuring specific drugs at designated intervals to maintain a constant concentration in a patient's bloodstream, thereby optimizing individual dosage regimens. The indications for drug monitoring include efficacy, compliance, drug-drug interactions, toxicity avoidance, and therapy cessation monitoring. Additionally, TDM can help to identify problems with medication compliance among noncompliant patient cases.

Biologic medicinal products (biologics) have transformed treatment landscapes worldwide for patients with haematological or solid malignancies with the 21st century. Today, as data exclusivity periods of first wave biologics approach expiration/have expired, several biosimilar products (i.e.,biologics that are considered to be similar in terms of quality, safety and efficacy to an approved 'reference' biologic) are being developed or have already been approved for human use.

Like all biologics, biosimilars are structurally complex proteins that are typicallymmanufactured using genetically engineered animal, bacterial or plant cell culture systems. As a consequence of this molecular complexity and the proprietary nature of the manufacturing process, which will inevitably result in the use of different host cell lines and expression systems as well as related differences in manufacturing conditions, it is not possible to manufacture exact copies of a reference biologic.

When administered to patients, all therapeutic proteins have the potential to induce an unwanted immune response (i.e.,tostimulate the formation of antidrug antibodies [ADAs]). The impact of immune responses can range from no apparent effect to changes in pharmacokinetics, loss of effect and serious adverse events. Furthermore, the immunogenicity profile of a biologic can be significantly altered by even small differences in its manufacturing process that are accompanied by a change in product attributes, as well as differences in dosing schedules, administration routes or patient populations.

SHIKARI® ELISA kits can be used for drug level and anti-drug antibodies measurements. SHIKARI® Ravulizumab ELISA products:

Brand	Description		Product Code
SHiKARi® (Q-RAV)	Ravulizumab	Free Drug	**RAV-FD-ULT**
SHiKARi® (S-ATRAV)	Ravulizumab	Antibody screening - Quantitative	**RAV-QNS-ULT**

Check the web page for the whole product list www.matriksbiotek.com

3. Test Principle

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and samples (serum or plasma) are incubated in the microtiter plate coated with the drug Ravulizumab. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds to Ravulizumab antibodies captured by the drug Ravulizumab on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The colour developed is proportional to the amount of Ravulizumab antibodies in the sample or standard. Results of samples can be determined directly using the standard curve.

4. Warnings and Precautions

- For professional use only.

- In case of severe damage of the kit package please contact Matriks Biotek® or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs but keep safe for complaint related issues.

- Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.

- Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further ınformation (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the local distributor.

- Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.

- All reagents of this kit containing human serum or plasma (standards etc.) have been tested and were found negative for HIV I/II, HBsAg and Anti-HCV. However, a presence of these or other ınfectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

- Reagents of this kit containing hazardous material may cause eye and skin irritations. See "Materials supplied", SDS and labels for details.

- Chemicals and prepared or used reagents must be treated as hazardous waste according the national biohazard safety guidelines or regulations.

5. Storage and Stability

The kit is shipped at ambient temperature (10-30°C) and should be stored at 2-8°C for long term storage. Keep away from heat or direct sunlight. The strips of microtiter plate are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8°C.

6. Specimen (Collection and Storage)

Serum, Plasma (EDTA, Heparin)

The usual precautions for venipuncture should be observed. Do not use grossly haemolytic, icteric or lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material. Avoid repeated freeze-thaw cycles for serum/plasma samples.

Samples should be diluted with the dilution rate given in the "Pre-test setup instructions" before the test.

Drug ınfusions may camouflages/mask the presence of antibody to drugs in serum/plasma samples. Therefore, blood sampling time is critical for detection of antibodies. It is recommended to take the blood sample just before the scheduled dose (trough specimen).

Storage	2-8°C	-20°C
Stability (serum/plasma)	2 days	6 months

7. Materials Supplied

Microtiter Plate	1 x 12 x 8	**Microtiter plate** Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with Ravulizumab.
Standard A-F	1 mL (each)	**Standards A-F** Standard A: 320 ng/mL Standard B: 160 ng/mL Standard C: 80 ng/mL Standard D: 40 ng/mL Standard E: 20 ng/mL Standard F: 0 ng/mL Ready to use. Used for the standard curve. Contains antibodies to Ravulizumab, human serum and stabilizer, <0,1% NaN_3.
Controls	1 mL (each)	**Control low and high levels** Ready to use. Contains human serum and stabilizer, <0,1% NaN_3. Control concentrations are given in "Quality control certificate"
Assay Buffer	1 x 50 mL	**Assay buffer** Ready to use. Blue coloured. Contains proteins, <0,1% NaN_3
Conjugate	1 x 12 mL	**Horse radish peroxidase conjugated probe** Ready to use. Red coloured. Contains HRP conjugated probe, stabilizer and preservatives.
Confirmation Reagent	1 x 12 mL	**Confirmation Reagent** Ready to use. Contains proteins, Ravulizumab and stabilizer. 0,1% NaN_3

Substrate	1 x 12 mL	**TMB substrate solution** Ready to use. Contains 3,3',5,5'-Tetramethylbenzidine (TMB).
Stop Buffer	1 x 12 mL	**TMB stop solution** Ready to use. 1N HCl.
Wash Buffer	1 x 50 mL	**Wash buffer (20x)** Prepared concentrated (20x) and should be diluted with the dilution rate given in the "Pre-test setup instructions" before the test. Contains buffer with tween 20.
Foil	2 x 1	**Adhesive Foil** For covering microtiter plate during incubation

8. Materials Required but Not Supplied

- Micropipettes and tips

- Calibrated measures

- Tubes for sample dilution

- Wash bottle, automated or semi-automated microtiter plate washing system

- Microtiter plate reader capable of measuring optical density with a photometer at OD 450nm with reference wavelength 650 nm (450/650 nm)

- Distilled or deionised water, paper towels, pipette tips and timer

9. Procedure Notes

- Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps must be performed strictly according to the instructions. Use calibrated pipettes and devices only.

- Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.

- Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.

- Use a pipetting scheme to verify an appropriate plate layout.

- Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an eight-channel micropipette for pipetting of solutions in all wells.

- Microplate washing is important. Improperly washed wells will give erroneous results. It isrecommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with wash buffer, and that there are no residues in the wells.

- Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. Pre-test Setup Instructions

- Preparation of components

Component	Wash buffer (Must be prepared before starting assay procedure)
Dilute	10 mL (e.g.)
With	Up to 200 mL
Diluent	Distilled water
Dilution Ratio	1/20
Remarks	Warm up 37°C to dissolve crystals. Mix vigorously
Storage	2-8°C
Stability	2 weeks

- Dilution of samples

Sample	Serum/Plasma
Diluent	Assay buffer
Dilution Ratio	1/10
Remarks	1/10 dilution 50 µL sample + 450 µL assay buffer

Patient samples with a concentration of drug above the measuring range are to be rated as > "Highest Standard (Standard A)". The result must not be extrapolated. The patient sample in question should be further diluted with assay buffer and retested.

- Preparation of confirmation test mixture

Sample	Serum/Plasma
Diluent	Confirmation Reagent
Dilution Ratio	1/10
Remarks	1/10 dilution 20 µL sample + 180 µL Confirmation Reagent

11. Test Procedure

	Total assay time: 140 minutes
1	Pipette 100 µL of each "Standards", "Low level control", "High level control" and diluted samples into the respective wells of microtiter plate **Wells** A1: Standard A B1: Standard B C1: Standard C D1: Standard D E1: Standard E F1: Standard F G1: Low level control H1: High level control A2 and on: Samples *It is advised to run more than one "Standard F (negative control)" samples for qualitative assay. Negative control studies can be duplicated or triplicated in order to take the mean value.
2	Cover the plate with adhesive foil. Briefly mix contents by gently shaking the plate. Incubate 60 minutes at room temperature (18-25°C).
3	Remove adhesive foil. Discard incubation solution. Wash plate three times each with 300 µL "Wash Buffer". Remove excess solution by tapping the inverted plate on a paper towel.

4	Pipette 100 µL "Conjugate" into each well.
5	Cover the plate with adhesive foil. Incubate 60 minutes at room temperature (18-25°C).
6	Remove adhesive foil. Discard incubation solution. Wash plate three times each with 300 µL "Wash Buffer". Remove excess solution by tapping the inverted plate on a paper towel.
7	Pipette 100 µL "Substrate" into each well
8	Incubate 20 minutes without adhesive foil at room temperature (18-25°C) in the dark.
9	Stop the substrate reaction by adding 100 µL "Stop Solution" into each well . Briefly mix contents by gently shaking the plate. Colour changes from blue to yellow.
10	Measure optical density with a photometer at OD 450nm with reference wavelength 650 nm (450/650 nm) within 30 minutes after pipetting the "Stop Solution"

Confirmation test for positive samples*
Prepare confirmation test mixture as described in "Pre-test Setup Instructions". Incubate this mixture for 60 minutes in a microtube *at room temperature (18-25°C)* After the incubation proceed the test procedure from step one given above by pipetting 100 µL of this solution to respective well. Instructions are followed givenin the test procedure in table. *It is recommended to run only positive samples for confirmation testing. Negative samples may give improper results in confirmation step.

12. Quality Control

The test results are only valid if the test has been performed following the instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. For a valid study, the OD 450/650 of the highest standard should be>1,000 and the OD 450/650 of the lowest standard should be <0,200. In case of any deviation the following technical issues (but not limited to) should be reviewed: Expiration dates of reagents, storage conditions, pipettes, devices, incubation conditions, washing methods, etc.

13. Calculation and Interpretation of Results

- Create a standard curve by using the standards. OD 450/650 nm for each standard on the vertical (Y-axis) axis versus the corresponding drug concentration on the horizontal (X-axis) axis.

- The concentration of the samples can be read directly from this standard curve. Using the absorbance value for each sample, determine the corresponding concentration of drug from the standard curve. Find the absorbance value on the Y-axis and extend a horizontal line to the curve. At the point of intersection, extend a vertical line to the X-axis and read the drug concentration of the unknown sample.

- If computer data is going to be used, we recommend primarily "FourParameter Logistic (4PL)" or secondly the "point-to-point calculation".

- To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (10x). Any sample reading greater than the highest standard should be further diluted appropriately with assay buffer and retested. Therefore, if the pre-diluted samples have been further diluted, the concentration determined from the standard curve must be multiplied by the further dilution factor.

e.g.; If the pre-diluted sample further diluted in a ratio of 1/10 then results should be multiplied by 100.

- For low and high level controls values, refer to “Quality Control Certificate” provided by each kit.

-Interpretation of true and false positives

$$\frac{\text{OD 450/650 sample} - \text{OD 450/650 sample with confirmation reagent}}{\text{OD 450/650 sample}} \times 100 = \text{inhibition \%}$$

if the inhibition is≥25% then the sample is “true positive”.

e.g.: If the OD 450/650 of the tested sample is 0,800 and after incubation of the sample with confirmation reagent and retested and the OD 450/650 of the sample is 0,200, then:

$$\frac{0{,}800 - 0{,}200}{0{,}800} \times 100 = 75\%$$

(the sample is “true positive” for anti-drug antibody)

- Qualitative interpretation

The results are evaluated by a cut-off value which is estimated by multiplying the mean OD 450/650 nm of the "standard F" by 3.

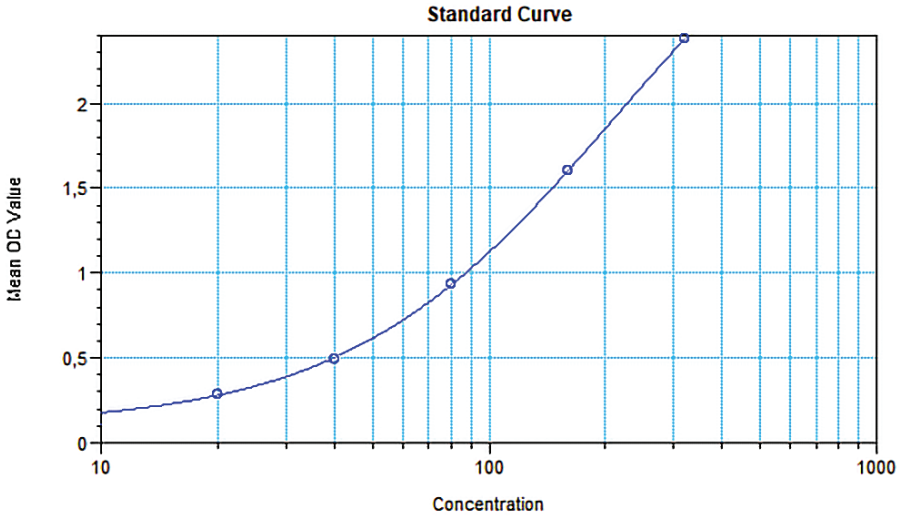
e.g. If "Sample OD 450/650 / the mean "standard F" OD 450/650 ⇢3" then the sample is POSITIVE

If "Sample OD 450/650 / the mean "standard F" OD 450/650 <3" then the sample is NEGATIVE

Note: The cut-off ınformation provided with this kit can only be considered as a recommendation. Cut-off values must be calculated/set or verified according to scientific standards by the users/laboratories.

14. Analytical Performance

- Calibration curve (Linearity, Dilutional linearity): r^2 >0,95



	y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
o	STD#1 (Standards: Concentration vs Mean OD Val...	0,101	1,254	211,74	3,74	1

This is only an example. Assay conditions will change in every assay and do not use this curve for your assay calculations.

- Sensitivity: The lowest detectable level (Lowest detection limit, LOD) that can be distinguished from the zero standard is 15 ng/mL

Functional sensitivity (Limit of quantification-LOQ): 20 ng/mL

- Specificity: There is no cross reaction with native serum immunoglobulin Recovery <100±30%.

- Precision: Intra-assay and inter-assay CVs <30%

- Cut-off: Cut-off values must be calculated/set or verified according to scientific standards by the users/laboratories.

The "Quality control certificate" contains lot specific analytical performance data and is supplied separately with each kit. If some further analytical performance data is needed, please refer to the local distributor.

15. Automation

SHIKARI® Quantitative Antibodies to Ravulizumab ELISA is also suitable to run on automated ELISA processors.

16. Symbols and Cautions

	Manufacturer		Temperature limitation
	Production date		See instruction for use
	Expiry date		Caution
LOT	Lot number	IVD	In vitro diagnostic medical device
REF	Catalog number	Control	Control
	Do not use if package is damaged	Control –	Negative control
	Keep away from sunlight	Control +	Positive control
	Keep dry		Number of tests

According to ISO 15223

Cautions: The performance of the kit can be achieved by fully complying with the instructions. Modifications on the test procedure can affect the results and these kinds of changes will not be charged as regular complaints. This product is for professional use only and must be used for "Intended use" that is given in the instructions for use. The results themselves should not be the only reason for any therapeutically consequences. They must be correlated to other clinical observations. Cut-off, reference ranges, etc. must be calculated/set according to scientific standards by the users/laboratories. Information in the instructions about cut-off, etc. performance characteristics, can only be considered as a recommendation and does not give any responsibility to the manufacturer.

Limitations of liability: The manufacturer's liability is limited to the purchase price of the product in all circumstances. The manufacturer cannot be held responsible for damage to the patient, lost profit, lost sales, damage to property or any other incidental or consequential loss.

Technical support and complaints: Technical support can be given upon request. If there is a problem with the product, complaints must be sent written to info@matriksbiotek.com with the technical data (if available) like standard curve, control results, etc. After the necessary examination, written reply will be given.

17. References

- Ladwig PM, Willrich MAV: Ravulizumab: Characterization and quantitation of a new C5 inhibitor using isotype specific affinity purification and high-resolution mass spectrometry. J Mass Spectrom Adv Clin Lab. 2021 Aug 12;21:10-18. doi: 10.1016/j.jmsacl.2021.08.002. eCollection 2021 Aug.

- Stern RM, Connell NT: Ravulizumab: a novel C5 inhibitor for the treatment of paroxysmal nocturnal hemoglobinuria. Ther Adv Hematol. 2019 Sep 10;10:2040620719874728.

- Syed YY: Ravulizumab: A Review in Atypical Haemolytic Uraemic Syndrome. Drugs. 2021 Apr;81(5):587-594.

- FDA Approved Drug Products: ULTOMIRIS (ravulizumab-cwvz) injection, for intravenous use

18. Revision summary

Revision no	Release date	Explanation
00	24.07.2024	New Documentation
01	26.09.2024	Section 11 has been revised.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