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''trace & catch''

Instructions for Use

SHIKARI® (S-ATDAR)

Qualitative Antibodies to Daratumumab ELISA

Enzyme immunoassay for determination of qualitative antibodies to Daratumumab in serum and plasma samples

REF	DAR-QLS-DAR
Σ	96 tests
	Shipment 10-30°C, Store 2-8°C
	MATRIKS BIOTECHNOLOGY CO., LTD. Gazi Universitesi Teknoplaza C Blok 10/50C/47 06830 Golbasi Ankara / TURKEY Tel +90 312 485 42 94 info@matriksbiotek.com
CE IVD	

1. Intended Use

SHIKARI® Qualitative Antibodies to Daratumumab ELISA has been especially developed for the qualitative analysis of antibodies to Daratumumab in serum and plasma samples. SHIKARI® Qualitative Antibodies to Daratumumab ELISA is optimized with Darzalex®.

2. General Information

Daratumumab is indicated as an intravenous injection alone or in combination with other medications for the treatment of multiple myeloma. Subcutaneous daratumumab with hyaluronidase is also indicated alone or in combination for the treatment of multiple myeloma.

Daratumumab is a monoclonal antibody that targets and induces apoptosis in cells that highly express CD38, including multiple myeloma cells. It has a long duration of action as it is given every 1-4 weeks. Patients should be counselled regarding the risk of hypersensitivity, neutropenia, thrombocytopenia, embryo-fetal toxicity, and interferences with cross-matching and red blood cell antibody screening.

CD38 is a glycoprotein present on the surface of hematopoietic cells and is responsible for a number of cell signalling functions. Daratumumab is an immunoglobulin G1 kappa (IgG1κ) monoclonal antibody that targets CD38. Cancers like multiple myeloma overexpress CD38, allowing daratumumab to have higher affinity for these cells. This binding allows daratumumab to induce apoptosis, antibody dependent cellular phagocytosis, and antibody and complement-dependent

cytotoxicity. Antibody dependent cellular phagocytosis is mediated by the FC region of the antibody inducing phagocytes such as macrophages, antibody dependent cellular cytotoxicity is mediated by the FC region of the antibody inducing effector cells such as natural killer cells, and complement dependent cytotoxicity is mediated by the FC region of the antibody binding to and inducing complement protein activity.

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

Brand	Description		Product Code
SHIKARI® (Q-DAR)	Daratumumab (Darzalex®)	Free Drug	DAR-FD-DAR
SHIKARI® (S-ATDAR)	Daratumumab (Darzalex®)	Antibody screening - Qualitative	DAR-QLS-DAR

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. Controls and samples (serum or plasma) are incubated in the microtiter plate coated with the drug Atezolizumab. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds to Daratumumab antibodies captured by the drug Atezolizumab on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. Finally, the reaction is terminated with an acidic stop solution. The colour developed

is proportional to the amount of Daratumumab antibodies in the sample or controls. The results can be evaluated with using cut-off value.

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 information (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 infectious agents can not 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", MSDS 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 infusions 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 Atezolizumab.
Controls	1 mL (negative) 0,3 mL (positive)	**Control Negative & Positive** Ready to use. Contains human serum and stabilizer, <0,1% NaN_3
Assay Buffer	1 x 12 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.
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 450 nm 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 is recommended 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 desicant.

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

11. Test Procedure

	Total assay time: 140 minutes
1	Pipette 100 µL "Assay Buffer" into each of the wells to be used.
2	Pipette 10 µL of each "Negative control", "Positive control" and samples into the respective wells of microtiter plate. Wells A1: Negative control* B1: Negative control* C1: Positive control D1 and on: Samples* It is advised to run more than one "Negative control" samples. Negative control studies can be duplicated or triplicated in order to take the mean value.

3	Cover the plate with adhesive foil. Briefly mix contents by gently shaking the plate. Incubate 60 minutes at room temperature (18-25°C).
4	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.
5	Pipette 100 µL "Conjugate" into each well.
6	Cover the plate with adhesive foil. Incubate 60 minutes at room temperature (18-25°C).
7	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.
8	Pipette 100 µL "Substrate" into each well.
9	Incubate 20 minutes without adhesive foil at room temperature (18-25°C) in the dark.
10	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.
11	Measure optical density with a photometer at OD 450 nm with reference wavelength 650 nm (450/650 nm) within 30 minutes after pipetting the "Stop Solution".

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 the run to be valid, the OD 450/650 nmof positive control should be >1,500 and the OD 450/650 nm of each negative control should be <0,150. 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

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

e.g.

If "Sample OD 450/650 / the mean negative control OD 450/650 ≥3"

then the sample is POSITIVE

If "Sample OD 450/650/ the mean negative control OD 450/650 <3"

then the sample is NEGATIVE

Note: The cut-off information 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

–Specificity: There is no cross reaction with native serum immunoglobulin

–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® Daratumumab 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 number		In vitro diagnostic medical device
	Catalog number		Control
	Do not use if package is damaged		Negative control
	Keep away from sunlight		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 can not 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

- VelchetiV, Viswanathan A, Govindan R. The proportion of patients with metastatic non-small cell lung cancer potentially eligible for treatment with Atezolizumab: A single institutional survey. J Thorac Oncol 2006 Jun;1(5):501.

- Church LD, McDermott MF: Atezolizumab, a fully-human mAb against IL-1beta for the potential treatment of inflammatory disorders. Curr Opin Mol Ther. 2009 Feb;11(1):81-9. [PubMed:19169963]

- Lachmann HJ, Kone-Paut I, Kuemmerle-Deschner JB, Leslie KS, Hachulla E, Quartier P, Gitton X, Widmer A, Patel N, Hawkins PN: Use of Atezolizumab in the cryopyrin-associated periodic syndrome. N Engl J Med. 2009 Jun 4;360(23):2416-25. doi: 10.1056/NEJMoa0810787. [PubMed:19494217]

- https://go.drugbank.com/drugs/DB09331

18. Revision summary

Revision no	Release Date	Explanation

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