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Instructions for Use

SHIKARI® (T-CAP NAb Assay-Nivolumab)

Target Capture Neutralizing Antibodies Immunoassay-Nivolumab

Enzyme immunoassay to detect neutralizing antibodies to Nivolumab in serum and plasma samples

REF	**NIV-TCAP-NAb-OPD**
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® Neutralizing Antibodies to Nivolumab ELISA (T-CAP NAb Assay-Nivolumab) has been especially developed for the neutralizing capability of antibodies to Nivolumab in serum and plasma samples. SHIKARI® Neutralizing Antibodies to Nivolumab ELISA is optimized with Opdivo®.

2. General Information

Biological therapeutic proteins can induce the production of anti-drug antibodies (ADA), including neutralizing antibodies (NAb), and consequently result in unwanted immune response in recipients.

NAb can diminish therapeutic efficacy by either preventing the drug from binding to its target or inhibiting downstream signaling upon binding due to steric hindrance. NAb can also cross-react and neutralize the biological activity of an endogenous counterpart in some cases, resulting in the impairment of an essential normal physiological function and life-threatening adverse effects. Therefore, it is essential to monitor the potential NAb during biological drug development. NAb monitoring provides the key information that could allow interpretation of safety studies. Non- neutralizing antibodies (non-NAb) are ADA that bind to sites on the drug molecule that do not affect target binding and thereby do not impact the drug's pharmacodynamic activity. Non-NAb are often referred to as 'binding antibodies', but that is an incorrect term because all types of ADA, are inherently binding antibodies since they bind drug as neutralizing antibodies. Neutralizing antibodies (NAb) are a subset of binding ADA that bind to the drug and inhibit its pharmacological function by preventing target binding. NAbs can inhibit drug activity soon after the drug is administered, but non-NAb do not inhibit the pharmacodynamic activity of the drug.

A neutralizing antibody assay is based on neutralizing antibodies' drug-target interaction blocking ability. As the concentration of NAb and/or NAb's binding affinity to drug gets higher, drug-target binding interaction gets lower. In Target Capture Competitive Ligand Binding NAb assays' design relies on the NAb and target's competition to bind to the drug between each other. NAb assays are named as direct binding when the presence of NAb can be detected by reduction in signal.

We recommend usage of SHIKARI® T-CAP NAb Assay with Quantitative ADA ELISA kits. First use Quantitative SHIKARI® ADA kit if the sample is positive then refer to T-CAP NAb Assay. Although SHIKARI® T-CAP NAb Assay can be used alone.

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 hematological 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 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 typically manufactured using genetically engineered animal, bacterial or plant cell culture systems. Because 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., to stimulate 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® Nivolumab ELISA products:

Brand	Description		Product Code
SHIKARI® (Q-NIVO)	Nivolumab	Free Drug	**NIV-FD-OPD**
SHIKARI® (S-ATN)	Nivolumab	Antibody screening - Qualitative	**NIV-QLS-OPD**
SHIKARI® (S-ATN)	Nivolumab	Antibody screening - Quantitative	**NIV-QNS-OPD**
SHIKARI® (T-CAP NAb ASSAY-NIVOLUMAB)	Nivolumab	Antibody screening - Neutralizing	**NIV-TCAP-NAb-OPD**

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

3. Test Principle

Target Capture Competitive Ligand Binding NAb assays' design relies on the NAb and target's competition to bind to the drug between each other. Controls and samples (serum or plasma) are incubated at room temperature with HRP conjugated Nivolumab. After 1 hour incubation the mixture transferred to the plate coated with target ligand protein of the drug. After incubation, the wells are washed, and chromogen-substrate is added to each well. Finally, the reaction is terminated with an acidic stop solution. The inhibition of the color development is proportional to the amount of neutralizing antibodies to Nivolumab in the sample or controls. The results can be evaluated by 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 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 to 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 hemolytic, 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)	7 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 target ligand protein of Nivolumab.
Controls	0,1 mL (negative) 0,1 mL (positive)	**Control negative and positive** Ready to use. Contains human serum and stabilizer, <0,05% procline.
Cut-Off Control	0,1 mL	**50 ng/ml Neutralizing Antibody Cut-Off Control** Ready to use. Contains human serum and stabilizer, <0,05% procline.
Non-Neutralizing Antibody Control	0,1 mL	**Non-Neutralizing Antibody Control** Ready to use. Contains stabilizer, <0,05% procline.
Conjugate (Capture Buffer)	1 x 50 mL	**Conjugate (Capture Buffer)** Ready to use. Red color. Contains 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 450nm with reference wavelength 650 nm (450/650 nm)

- Distilled or deionized 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 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

Preparation of Positive Control, Negative Control, Cut off, Non-Neutralizing Antibody Control and Samples

Sample	Positive Control, Negative Control Cut-off, Non-Neutralizing Antibody Control	Serum/Plasma
Conjugate (Capture Buffer)	Conjugate (Capture Buffer)	Conjugate (Capture Buffer)
Dilution Ratio	1:20	1:20
Remarks	1:20 mixture 12,5 µL controls + 237,5 µL conjugate = 250 µL mixed controls	1:20 mixture 10 µL sample + 190 µL conjugate = 200 µL mixed sample

11. Test Procedure

	Total assay time: 140 minutes
1	Prepare mixtures with each of the samples and controls as described in the "Pre-Test Setup Instructions" section. Incubate at room temperature for 60 min.
2	Pipette 100 µL of each "Positive control", "Negative control", "Cut-Off Control", ''Non-Neutralizing Antibody Control'' and "Mixed Samples" into the respective wells of microtiter plate. **Wells** A1: Positive control B1: Negative control C1: Negative control D1: Cut-Off control E1: Cut-Off control F1: Non-Neutralizing Antibody Control G1: Non-Neutralizing Antibody Control H1 and on: Mixed Samples
3	Cover the plate with adhesive foil. Briefly mix the 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 "Substrate" into each well.
6	Incubate 20 minutes without adhesive foil at room temperature (18-25°C) in the dark.
7	Stop the substrate reaction by adding 100 µL "Stop Solution" into each well. Briefly mix the contents by gently shaking the plate. Color changes from blue to yellow.
8	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, as this is an inhibition assay, the OD 450/650 nm of positive control should be equal or less than OD value of Cut-Off control OD value and the OD 450/650 nm of negative control should be greater than OD value of Cut-Off control OD values'. 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. Interpretation of Results

Negative control and non-neutralizing antibody control does not contain neutralizing ADA. Cut-off control contains neutralizing ADA, positive control contains with the known amount of neutralizing ADA. OD value of Cut-Off control indicates limit of detection for the kit. OD values below the mean Cut-off OD value interpreted as positive meaning sample contains NAb. OD values of the Non-Neutralizing Antibody Control should be above the value of Cut-off OD similar to Negative Control.

14. Analytical Performance

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

- Cut-off: Cut-off control supplied as separate reagent 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® Neutralizing Antibodies to Nivolumab 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 techsupport@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

- Gunn, G. R., 3rd, Sealey, D. C., Jamali, F., Meibohm, B., Ghosh, S., & Shankar, G. (2016). From the bench to clinical practice: Understanding the challenges and uncertainties in immunogenicity testing for biopharmaceuticals. Clinical and experimental immunology, 184(2), 137–146

- Shankar G, Arkin S, Cocea L et al Assessment and reporting of the clinical immunogenicity of therapeutic proteins and peptides-harmonized terminology and tactical recommendations. AAPS J 2014; 16:658–73.

- Rathi A, Rinker S, Niu H, Carter C, Kumar S, Cowan K. Assay development considerations to improve drug tolerance in direct competitive ligand binding neutralizing antibody assays, pretreatment strategies. J Immunol Methods. 2023 Jun;517:113484

- Wang, J., Lozier, J., Johnson, G. et al. Neutralizing antibodies to therapeutic enzymes: considerations for testing, prevention and treatment. Nat Biotechnol 26, 901–908 (2008)

- Wu, B., Chung, S., Jiang, XR. et al. Strategies to Determine Assay Format for the Assessment of Neutralizing Antibody Responses to Biotherapeutics. AAPS J 18, 1335–1350 (2016).

18. Revision summary

Revision no	Release date	Explanation
00	15.05.2024	New Documentation

Notes:


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