MATRIKS BIOTEK
innovation for health & wellness

"trace & catch"

Instruction for Use

Antibody to Nivolumab (Opdivo®)

SHIKARI®
S-ATN

Enzyme immunoassay for the quantitative determination (screening) of antibodies to nivolumab (Opdivo®) in serum and plasma

REF **TR-ATNv1** 12 x8 2-8 C

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IVD

Matriks Biotek® Laboratories
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	SHIKARI S-ATN
	Nivolumab (Opdivo®) antibodies qualitative analyse
Required Volume (µl)	10
Total Time (min)	140
Sample	Serum, plazma
Sample Number	96
Detection Limit (ng/mL)	+/-
Spike Recovery (%)	-
Shelf Life (year)	1

Intended Use

The Matriks Biotek® Antibody to nivolumab (Opdivo®) Enzyme-Linked-ImmunoSorbent-Assay (ELISA) Kit is intended for the qualitative determination of antibodies to nivolumab (Opdivo®) in serum and plasma. It is for professional use only.

Summary and Explanation

According to prospectus; nivolumab (OPDIVO®) is a programmed death receptor-1 (PD-1) blocking antibody indicated for the treatment of patients with:

Unresectable or Metastatic Melanoma
OPDIVO® (nivolumab) as a single agent is indicated for the treatment of patients with BRAF V600 wild-type unresectable or metastatic melanoma.
OPDIVO® (nivolumab) as a single agent is indicated for the treatment of patients with BRAF V600 mutation-positive unresectable or metastatic melanoma.
This indication is approved under accelerated approval based on progression-free survival. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.
OPDIVO® (nivolumab), in combination with ipilimumab, is indicated for the treatment of patients with unresectable or metastatic melanoma.
This indication is approved under accelerated approval based on progression-free survival. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

Metastatic Non-Small Cell Lung Cancer
OPDIVO® (nivolumab) is indicated for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) with progression on or after platinum-based chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving OPDIVO®.

Renal Cell Carcinoma
OPDIVO® (nivolumab) is indicated for the treatment of patients with advanced renal cell carcinoma (RCC) who have received prior anti-angiogenic therapy.

Classical Hodgkin Lymphoma
OPDIVO® (nivolumab) is indicated for the treatment of patients with classical Hodgkin lymphoma (cHL) that has relapsed or progressed after autologous hematopoietic stem cell transplantation (HSCT) and post-transplantation brentuximab vedotin. This indication is approved under accelerated approval based on overall response rate. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

Test Principle

The Matriks Biotek® Antibody to nivolumab (Opdivo®) ELISA is a sandwich assay for the determination of antibodies against nivolumab in serum and plasma samples.
During the first incubation period, antibodies to nivolumab (ATN) in patient serum/ plasma samples are captured by the drug nivolumab (Opdivo®) coated on the wall of the microtiter wells. After washing away the unbound components from samples, a peroxidase-labelled specific conjugate is added to each well and then incubated.
After a second washing step, the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen-substrate. Finally, the reaction is terminated with an acidic stop solution. The intensity of the reaction color is directly proportional to the concentration of ATN in sample.

Warnings and Precautions

1. For professional use only.

2. 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.

3. 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.

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

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

6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.

7. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guidelines or regulations.

8. Avoid contact with Stop solution. It may cause skin irritations and burns.

9. Some reagents contain sodium azide (NaN3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with large volume of water to avoid azide build-up.

10. All reagents of this test kit containing human serum or plasma have been tested and were found negative for HIV I/II, HBsAg and HCV by FDA approved procedures. 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.

Storage and Stability

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters. The strips of microtiter plate is stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

Specimen Collection and Storage

Serum, Plasma (EDTA, Heparin)*

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	-20°C	Keep away from heat or direct sun light
Stability:	7 d	6 mon	Avoid repeated freeze-thaw cycles

*. Nivolumab (Opdivo®) infusion camouflages/masks the presence of antibody to Nivolumab in serum/plasma samples. Therefore, blood sampling time is critical for detection of nivolumab. Matriks Biotek® Laboratories propose to obtain blood sample just before the infusion of nivolumab (Opdivo®) or at least 2 weeks after the infusion of nivolumab (Opdivo®).

Materials Supplied

1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with nivolumab.
1 x 0.25 mL	RCTV CNTR	**Reactive Control** Ready-to-use. Contains nivolumab -reactive reagent, human serum, stabilizers and <0.1% NaN_3
1 x 0.5 mL	NEG CNTR	**Negative Control** Ready-to-use. Contains human serum, stabilizers and <0.1% NaN_3
1 x 12 mL	ASSAY BUF	**Assay Buffer** Blue colored. Ready to use. Contains proteins and <0.1% NaN3
1 x 12 mL	POD CONJ	**Peroxidase Conjugate** Red colored. Ready to use. Contains peroxidase (POD) conjugate, RF blockers, stabilizer and preservatives.
1 x 12 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains TMB
1 x 12 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1N HCl.
1 x 50 mL	WASHBUF CONC	**Wash Buffer, Concentrate (20x)** Contains Buffer with Tween 20.
2 x 1	ADH FILM	**Adhesive Film** For covering of Microtiter Plate during incubation.

Materials Required but not Supplied

1. Micropipettes (< 3% CV) and tips to deliver 5-1000 µL.
2. Bidistilled or deionised water
3. Calibrated measures.
4. Absorbent paper and timer.
5. Standard laboratory glass or plastic vials, cups, etc.
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 650 nm is optional).

Procedure Notes

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

2. 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.

3. 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.

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

5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.

6. 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.

7. 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.

Preparation of Component

Dilute/ disolve	Component	with	Diluent	Rela-tion	Remarks	Storage	Stability
10 mL	Wash Buffer*	Up to 200 mL	Bidist. water	1:20	Warm up at 37°C to dissolve crystals. Mix vigorously.	2-8 °C	3 w

*. Prepare Wash Buffer before starting assay procedure.

Test Procedure

1	Pipette 100µl of Assay Buffer non-exceptionally into each of the wells to be used.
2	**QUALITATIVE ELISA TEST FORMAT** Pipette 10 µL of ready-to use Cut-Off Serum, Reactive Control, and Samples into the respective wells of microtiter plate. Wells A1: Negative Control B1: Negative Control C1: Reactive Control D1 and on:. Sample (Serum/Plasma)
3	Cover the plate with adhesive film. Briefly mix contents by gently shaking the plate. Incubate 60 min at room temperature (18-25°C).
4	Remove adhesive film. Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
5	Pipette 100 µL of ready-to use Peroxidase Conjugate into each well.
6	Cover the plate with adhesive film. Incubate 60 min at room temperature (18- 25°C).
7	Remove adhesive film. Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
8	Pipette 100 µL of TMB Substrate Solution into each well.
9	Incubate 20 min (without adhesive foil.) at room temperature (18-25°C) in the dark.
10	Stop the substrate reaction by adding 100 µL of Stop Solution into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow
11	Measure optical density with a photometer at 450/650 nm within 30 min after pipetting of the Stop Solution.

Interpretation Of Results

For the run to be valid, the OD_{450} nm of Positive Control should be ≥ 1.00 and the OD450/650 nm of each Negative Control should be <0.200 0, if not, improper technique or reagent deterioration may be suspected and the run should be repeated.

The results are evaluated by a cut-off value which is estimated by multiplying the mean OD450/650 nm of the negative controls by 3.

I.e.;

If "Sample $OD_4$450/650 the mean OD450 /650 of Negative Controls" is ≥3, the sample is POSITIVE If "Sample OD450/650 the mean OD450/650 of Negative Controls" is <3, the sample is NEGATIVE

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