MATRIKS BIOTEK
innovation for health & wellness

"trace & catch"

Instructions for Use

Pembrolizumab (Keytruda®) ELISA

SHIKARI®

Q-PEM

Enzyme immunoassay for the quantitative determination of Pembrolizumab (Keytruda®) in serum and plasma

REF **TR-PEMv1** Σ 12 x 8 2-8°C

CE IVD

Matriks Biotek® Laboratories
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	SHIKARI® Q-PEM
	Pembrolizumab (Keytruda®) quantitative analyses
Required Volume (µl)	10
Total Time (min)	140
Sample	Serum, plasma
Sample Number	96
Detection Limit (µg/mL)	0,03
Spike Recovery (%)	Between 80-120
Shelf Life (year)	1

Intended Use

Enzyme immunoassay for the quantitative determination of pembrolizumab (Keytruda®) in serum and plasma. *Matriks Biotek® pembrolizumab ELISA* has been especially developed for the quantitative analysis pembrolizumab in serum and plasma samples between the Cmin and Cmax range of concentrations indicated in the pharmacokinetics section of prospectus.

Summary and Explanation

According to the prospectus; KEYTRUDA is a programmed death receptor-1 (PD-1)-blocking antibody indicated for the treatment of:

-patients with unresectable or metastatic melanoma.

-patients with metastatic NSCLC whose tumors have high PD-L1 expression [(Tumor Proportion Score (TPS) ≥50%)] as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations, and no prior systemic chemotherapy treatment for metastatic NSCLC.

-patients with metastatic NSCLC whose tumors express PD-L1 (TPS ≥1%) as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving KEYTRUDA.

-patients with recurrent or metastatic HNSCC with disease progression on or after platinum-containing chemotherapy. This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

- adult and pediatric patients with refractory cHL, or who have relapsed after 3 or more prior lines of therapy. This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

1.1 Melanoma

KEYTRUDA® (pembrolizumab) is indicated for the treatment of patients with unresectable or metastatic melanoma .

1.2 Non-Small Cell Lung Cancer

KEYTRUDA is indicated for the first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have high PD-L1 expression [Tumor Proportion Score (TPS)≥50%)] as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations.

KEYTRUDA is indicated for the treatment of patients with metastatic NSCLC whose tumors express PD-L1 (TPS≥1%) as determined by an FDA-approved test, with disease progressşon on or after platinum containing chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving KEYTRUDA.

1.3 Head and Neck Cancer

KEYTRUDA is indicated for the treatment of patients with recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) with disease progression on or after platinum-containing chemotherapy.
This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

1.4 Classical Hodgkin Lymphoma

KEYTRUDA is indicated for the treatment of adult and pediatric patients with refractory classical Hodgkin lymphoma (cHL), or who have relapsed after 3 or more prior lines of therapy. This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

Test Principle

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and samples (serum or plasma) are incubated in the microtitre plate coated with the reactant specific for Pembrolizumab (Keytruda®). Following incubation wells are washed and then horse radish peroxidase (HRP) conjugate is added and binds to Pembrolizumab. After incubation, the wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. The color developed is proportional to the amount of Pembrolizumab (Keytruda®) in the sample or standard. Results of samples can be determined directly using the standard curve.

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 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. All reagents of this kit containing human serum (i.e. standards) have been tested and were found negative for HIV I/II, HBsAg and 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.

10. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 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.

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 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 Avoid repeated freeze-thaw cycles
Stability:	2 d	6 mon	

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

Materials Supplied

1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with reactant for detection of Pembrolizumab (Keytruda®).
7 x 0.3 mL	STND A-E HIGH CNTRL LOW CNTRL	**Pembrolizumab Standards A-E, High Level Control, Low Level Control** 1; 0,3; 0,1; 0.03 and 0 microgram/mL Ready to use. Used for construction of the standard curve. Contains pembrolizumab (Keytruda®), human serum, stabilizer and <0.1% NaN_3.
1 x 50 mL	ASSAY BUF	**Assay Buffer** Blue colored. Ready to use. Contains proteins and <0.1% NaN_3.
1 x 12 mL	HRP CONJ	**Peroxidase Conjugate** Red colored. Ready to use. Contains horse radish peroxidase (HRP) and stabilizers.
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.
2x1	SLGP	**Semi-Log Graph Paper** For constructing standard curve and calculation of results

Materials Required but not Supplied

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV).
2. Calibrated measures.
3. Tubes for sample dilution.

4. Wash bottle, automated or semi-automated microtiter plate washing system
5. Microtiter plate reader capable of reading absorbance at 450 nm.
6. Bidistilled or deionised water, paper towels, pipette tips and timer.

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 micropipette 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.

Pre-Test Setup Instructions

1. Preparation of Components

Dilute/ disolve	Component	with	Diluent	Relation	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	4 w

***.** Prepare Wash Buffer before starting assay procedure.

2. Dilution of Samples*

Sample	To be diluted	With	Relation	Remarks
Serum/ Plasma	Initially 1:100	Assay Buffer	1:100	For dilution at 1:100; 10µl Sample + 990µl Assay Buffer

*. Patient samples with a concentration of pembrolizumab (Keytruda®) above the measuring range are to be rated as "> highest standard". The result must not be extrapolated. The patient sample in question ***should be diluted with assay buffer*** and retested.

Test Procedure

1	Pipette 100µl of Assay Buffer non-exceptionally into each of the wells to be used.
2	Pipette 10 µL of each ready-to use Standards, High level control, Low 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: High Level Control G1: Low Level Control H1 and on: Sample (Serum/Plasma)
3	Cover the plate with adhesive foil. Incubate 60 min at room temperature (18- 25°C).
4	Remove adhesive foil. 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 HRP-Conjugated Probe into each well.
6	Cover the plate with adhesive foil. Incubate 60 min at room temperature (18- 25°C).
7	Remove adhesive foil. 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.

Quality Control

The test results are only valid only 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. All standards must be found within the acceptable ranges as stated above and/or label. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

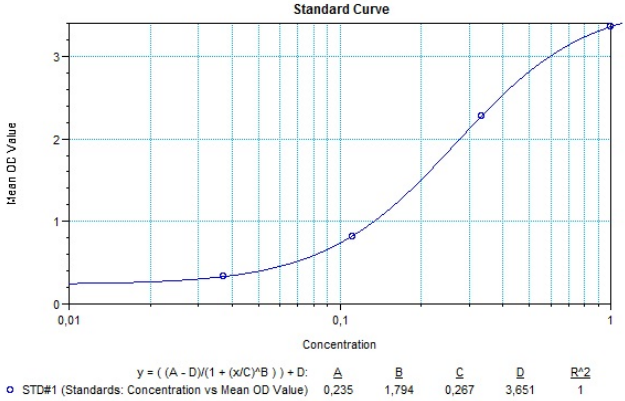
Calculation & Interpretation of Results

1. A standard curve should be calculated using the standard concentration (X-axis) versus the OD450 (we suggest that OD readings done at double wavelength at OD450/650 and OD value read at 650nm subtracted from 450nm OD VALUE) VALUES (Y-axis).In case of manual plot, we suggest semilog graph paper. If computer data regation is going to be used, we recommend primarily "4 Parameter Logistic (4PL)" or secondly the "point-to-point calculation".

2. The concentration of the samples can be read from this standard curve as follows. Using the absorbance value for each sample, determine the corresponding concentration of the drug from standard curve. This value always has to be multiplied by the dilution factor. If any diluted sample is reading greater than highest standard, it should be further diluted appropriately with Assay Buffer and retested. Also this second dilution has to be used for calculation the final results.

3. Any sample diluted at 1:100 and still reading greater than the highest standard should be further diluted appropriately with Assay Buffer and retested. **Because the samples have been diluted, the concentration determined from the standard- curve must be multiplied by the dilution factor.**

Typical Calibration Curve
(Example. Do not use for calculation!)



Standard	Concentration (µg/mL)	Mean OD450/650
A	1	3,359
B	0.3	2,277
C	0.1	0,821
D	0.03	0,331
E	0	0,029

Assay Characteristics

1. **Specificity:** There is no cross reaction with native serum immunoglobulins and tested monoclonal antibodies such as infliximab (Remicade®) and/or adalimumab (Humira®) , etanercept (Enbrel®), bevacizumab (Avastin®), trastuzumab (Herceptin®)

2. **Sensitivity:** The lowest detectable level that can be distinguished from the zero standard is 0,010 µg/mL.

3. **Precision Of Kit:**
   Intra-assay CV: <20% for pembrolizumab range 0.01-1 µg/mL.
   Inter-assay CV: <20% for pembrolizumab range 0.01-1 µg/mL.

4. **Recovery:** Recovery rate was found to be between 80-120% with normal human serum samples supplemented with known concentrations of pembrolizumab.

Automation

Experiments have shown that the Pembrolizumab ELISA is also suitable to run on an automated ELISA processor.

References

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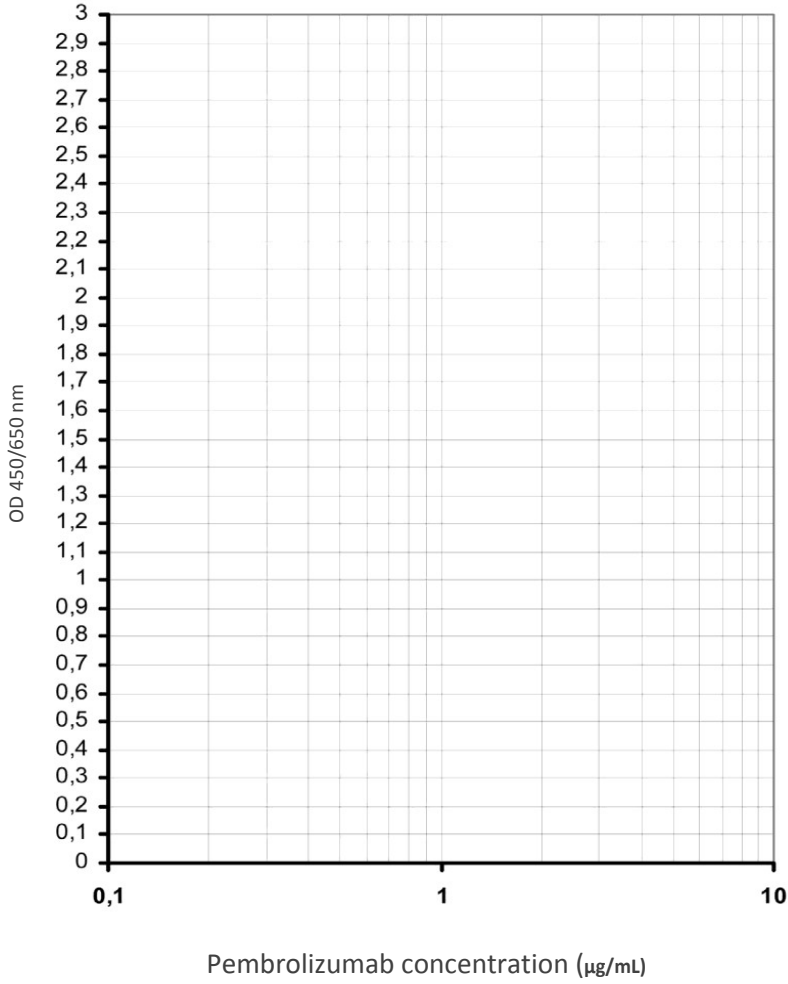
OD 450/650 nm
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2,5
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0,1
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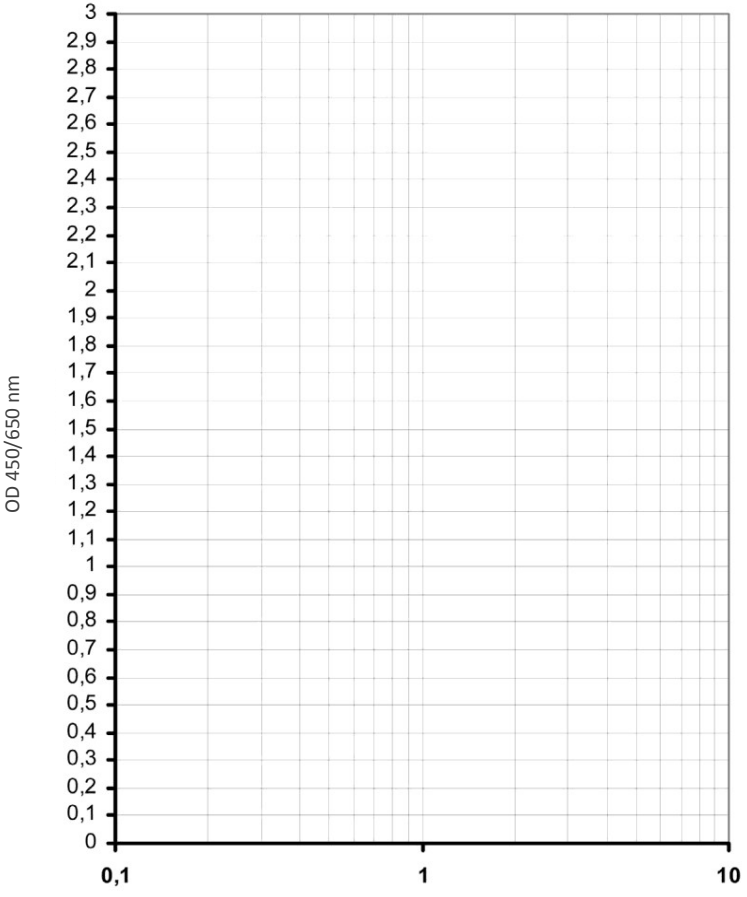
10
Pembrolizumab concentration (µg/mL)


OD 450/650 nm
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Pembrolizumab concentration (µg/mL)