

BioSim™ anti-Pembrolizumab (Keytruda®) (Human) ELISA Kit 10/17

(Catalog # E4397-100, 100 assays, Store at 4°C)

I. Introduction:

Pembrolizumab (Keytruda®) is an antibody drug that targets the cell surface receptor programmed cell death protein 1 (PD-1) found on T cells. By preventing the binding of its ligands (PD-L1 and PD-L2), pembrolizumab induces an antitumor immune response. Upregulation of PD-1 ligands is a mechanism for tumors to evade antitumor immune response; when PD-1 binds its ligand, the T cell receives an inhibitory signal leading to T cell anergy and blockade of anti-tumor immune response. Instead of directly targeting tumor tissue to induce tumor cell death, pembrolizumab acts as a checkpoint inhibitor to stimulate immune responses to eliminate cancer cells. However, some patients develop unwanted immunogenicity, which leads to production of anti-drug-antibodies (ADAs) inactivating the therapeutic effects of the treatment and, in rare cases, inducing adverse effects. BioVision's BioSim™ anti-Pembrolizumab ELISA kit is designed to detect antibody against Pembrolizumab with high specificity and sensitivity in biological matrices.

II. Application:

This ELISA kit is used for *in vitro* qualitative determination of antibody against Pembrolizumab in serum and plasma
 Cross Reactivity: Pembrolizumab (Keytruda®) infusion camouflages/masks the presence of antibody to Pembrolizumab (ATP) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATP. It is convenient to obtain blood sample just before the infusion or at least 2 weeks after the infusion of Pembrolizumab.

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4397-100	Part No.
Micro ELISA Plate	1 plate	E4397-100-1
Positive Control	0.25 ml	E4397-100-2
Negative Control	0.5 ml	E4397-100-3
Assay Buffer	50 ml	E4397-100-4
Peroxidase Conjugate	12 ml	E4397-100-5
TMB substrate (Avoid light)	12 ml	E4397-100-6
Stop Solution	12 ml	E4397-100-7
Wash buffer (20X)	50 ml	E4397-100-8
Plate sealers	2	E4397-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment.

VII. Reagent and Sample Preparation:

Note: Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer:** Dilute the 20X Wash Buffer to 1X solution in ddH₂O (10 ml of Wash Buffer stock to 190 ml of ddH₂O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.
2. **Samples preparation:** The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 7 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

VIII. Assay Protocol:

Note: Bring all reagents, microplate and samples to room temperature 15 minutes prior to the assay.

It is recommended that all standards and samples be run at least in duplicate.

A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Pipette 100 µl of **Assay Buffer** into each of the wells to be used.
3. Add 10 µl of **negative control** (2 wells), **positive control**, and **samples** into appropriate wells. Cover wells and incubate for 60 minutes at room temperature (RT).
4. 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. Add 100 µl of **Peroxidase Conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 60 minutes.
6. Discard the solution and wash the wells as step 3.
7. Add 100 µl of 1X **TMB substrate** solution and incubate the plate in dark at RT for 20 minutes

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8. Add 100 μ l of **Stop solution** to stop the reaction

9. Read the absorbance in micro plate reader set to 450 nm within 20 minutes. (reference wavelength to 650 nm)

IX. QUALITATIVE INTERPRETATION:

- For the run to be valid, the OD_{450/650} nm of Positive Control (Standard A) should be ≥ 1.0 and the OD_{450/650} nm of each Negative Control should be <0.2 , if not, improper technique or reagent deterioration may be suspected and the run should be repeated.
- If “Sample OD_{450/650} / Negative Control OD_{450/650}” is < 3 , the sample is NEGATIVE for Antibody to Pembrolizumab.
- If “Sample OD_{450/650} / Negative Control OD_{450/650}” is ≥ 3 , the sample is POSITIVE for Antibody to Pembrolizumab.

X. RELATED PRODUCTS:

- BioSim™ Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4371-100)
- BioSim™ Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4372-100)
- BioSim™ Bevacizumab (Avastin®) (Human) ELISA Kit (Cat. No. E4373-100)
- BioSim™ Etanercept (Enbrel®) (Human) ELISA Kit (Cat. No. E4374-100)
- BioSim™ Infliximab (Remicade®) (Human) ELISA Kit (Cat. No. E4375-100)
- BioSim™ Trastuzumab (Herceptin®)(Human) ELISA Kit (Cat. No. E4376-100)
- BioSim™ Golimumab (Simponi®)(Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Infliximab (Remsima®)(Human) ELISA Kit (Cat. No. E4378-100)
- BioSim™ Cetuximab (Erbitux®)(Human) ELISA Kit (Cat. No. E4379-100)
- BioSim™ Denosumab (Prolia®)(Human) ELISA Kit (Cat. No. E4380-100)
- BioSim™ Omalizumab (Xolair®)(Human) ELISA Kit (Cat. No. E4381-100)
- BioSim™ Nivolumab (Opdivo®)(Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Keytruda®)(Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Yervoy®)(Human) ELISA Kit (Cat. No. E4384-100)
- BioSim™ Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4385-100)
- BioSim™ Trastuzumab (Herceptin®) (Human) ELISA Kit (Cat. No. E4386-100)
- BioSim™ Infliximab (Remicade®) (Human) ELISA Kit (Cat. No. E4387-100)
- BioSim™ Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4388-100)
- BioSim™ Bevacizumab (Avastin®) (Human) ELISA Kit (Cat. No. E4389-100)
- BioSim™ Infliximab (Remsima®) (Human) ELISA Kit (Cat. No. E4390-100)
- BioSim™ Cetuximab (Erbitux®) (Human) ELISA Kit (Cat. No. E4391-100)
- BioSim™ Etanercept (Enbrel®) (Human) ELISA Kit (Cat. No. E4392-100)
- BioSim™ Golimumab (Simponi®) (Human) ELISA Kit (Cat. No. E4393-100)
- BioSim™ Denosumab (Prolia®) (Human) ELISA Kit (Cat. No. E4394-100)
- BioSim™ Omalizumab (Xolair®) (Human) ELISA Kit (Cat. No. E4395-100)
- BioSim™ Nivolumab (Opdivo®) (Human) ELISA Kit (Cat. No. E4396-100)
- BioSim™ Pembrolizumab (Keytruda®) (Human) ELISA Kit (Cat. No. E4397-100)
- BioSim™ Ipilimumab (Yervoy®) (Human) ELISA Kit (Cat. No. E4398-100)
- BioSim™ Filgrastim (Herceptin®) (Human) ELISA Kit (Cat. No. E4399-100)

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