ab237652 – Pembrolizumab ELISA Kit

For the quantitative analysis of free pembrolizumab in human serum and plasma samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab237652

Storage and Stability

On receipt entire assay kit should be stored at +4°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer	2 x 50 mL	+4°C
HRP-conjugate Probe	12 mL	+4°C
Micro ELISA Plate	1 unit	+4°C
Pembrolizumab Standard S1	0.3 mL	+4°C
Pembrolizumab Standard S2	0.3 mL	+4°C
Pembrolizumab Standard S3	0.3 mL	+4°C
Pembrolizumab Standard S4	0.3 mL	+4°C
Pembrolizumab Standard S5	0.3 mL	+4°C
Pembrolizumab Standard S6	0.3 mL	+4°C
Pembrolizumab Standard S7	0.3 mL	+4°C
Plate sealers	2 units	+4°C
Stop Solution	12 mL	+4°C
TMB substrate	12 mL	+4°C
Wash Buffer (10X)	50 mL	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.
- Before using the kit, spin the tubes prior to opening.

Wash Buffer: Dilute the 10X Wash Buffer to 1X solution in ddH2O (10 ml of Wash Buffer stock to 90 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.

Standard Preparation: Ready to use.

Standard	S1	S2	S3	S4	S5	S6	S7
Concentration (ng/ml)	1000	300	100	30	0	High Control	Low Control

Sample Dilution

- **Serum/Plasma:** Dilute samples 1/100 (10 µl Sample + 990 µl Assay Buffer).
- Diluted samples should further be diluted if the concentration of Pembrolizumab is higher than the measuring range.
- The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 2 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

Assay Protocol

- 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 the Reagent Preparation section.
2. Pipette 100 µl of Assay Buffer non-exceptionally into each of the wells to be used.
3. Add 10 µl of standards, controls and diluted-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 HRP-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 4.
7. Add 100 µl of 1X TMB substrate solution and incubate the plate in dark at RT for 20 minutes.
8. Add 100 µl 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).

Calculation

Using the standards disregarding zero standard, construct a standard curve by plotting the OD450/650 nm for each of 4 standards on the Y-axis versus the corresponding Pembrolizumab concentration on the X-axis. Construct a standard curve of difference data using software capable of generating four parameter logistic (4PL) or point-to-point calculation curve fit. To obtain the exact values of the samples, the concentration determined from the standard-curve should be multiplied by the dilution factor.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)