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Anti-Pembrolizumab Neutralizing antibody ELISA Kit

ADMEbio Catalog Number: EKFT19741UV **For Research Use Only.** Not for use in diagnostic procedures.

Product Overview

Name	Anti-Pembrolizumab Neutralizing antibody ELISA Kit
Reactivity	Universal
Sensitivity	0.94 ng/ml
Assay Range	1.56-100 ng/ml
Detection Method	Competitive ELISA, Coated with Antigen
Sample type(s)	Serum, Plasma, Cell Culture Supernatant, cell or tissue lysate, Other liquid samples

Product Features			
Application	In vitro quantitative determination of Pembrolizumab NAb concentrations in serum, plasma, cell culture supernatant and other biological samples.	In vitro quantitative determination of Pembrolizumab NAb concentrations in serum, plasma, cell culture supernatant and other biological samples.	In vitro quantitative determination of Pembrolizumab NAb concentrations in serum, plasma, cell culture supernatant and other biological samples.
Detection Duration	2 hours(excluding balancing and sample preparation)	2 hours(excluding balancing and sample preparation)	2 hours(excluding balancing and sample preparation)
Samples needed for single well(Max)	Serum: 50ul, Plasma: 50ul, Cell Culture Supernatant: 50ul, cell or tissue lysate: 50ul, Other liquid samples: 50ul	Serum: 50ul, Plasma: 50ul, Cell Culture Supernatant: 50ul, cell or tissue lysate: 50ul, Other liquid samples: 50ul	Serum: 50ul, Plasma: 50ul, Cell Culture Supernatant: 50ul, cell or tissue lysate: 50ul, Other liquid samples: 50ul
Specificity	Specifically recognize Pembrolizumab NAb, no obvious cross reaction with other analogues	Specifically recognize Pembrolizumab NAb, no obvious cross reaction with other analogues	Specifically recognize Pembrolizumab NAb, no obvious cross reaction with other analogues
Storage	2-8°C (for sealed box), please do not freeze! See kit label for expiry date	2-8°C (for sealed box), please do not freeze! See kit label for expiry date	2-8°C (for sealed box), please do not freeze! See kit label for expiry date

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Principle of the Assay

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with PD-1. During the reaction, Pembrolizumab NAb in the sample or standard competes with a fixed amount of PD-1 on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to Pembrolizumab. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm. The concentration of Pembrolizumab NAb in the samples is then determined by comparing the OD of the samples to the standard curve. The concentration of the target substance was inversely proportional to the OD450 value.

Kit Components and Storage

The sealed kit can be stored at 2-8 ℃. The storage condition for opened kit is specified in the table below:

Item	Size(48T)	Size(96T)	Storage Condition for Opened Kit
ELISA Microplate(Dismountable)	8×6	8×12	Put the rest strips into a sealed foil bag with the desiccant. Stored for 1 month at 2-8°C; Stored for 12 month at -20°C
Lyophilized Standard	1vial	2vial	Put the rest standards into a desiccant bag. Stored for 1 month at 2-8°C; Stored for 12 month at -20°C
Biotin-labeled Antibody(Concentrated, 100X)	30ul	60ul	2-8°C (Avoid Direct Light)
HRP-Streptavidin Conjugate(SABC, 100X)	60ul	120ul	2-8°C (Avoid Direct Light)
TMB Substrate	5ml	10ml	2-8°C (Avoid Direct Light)
Sample Dilution Buffer	10ml	20ml	2-8°C
Antibody Dilution Buffer	5ml	10ml	2-8°C
SABC Dilution Buffer	5ml	10ml	2-8°C
Stop Solution	5ml	10ml	2-8°C
Wash Buffer(25X)	15ml	30ml	2-8°C
Plate Sealer	3 pieces	5 pieces	

Product Description	1 copy	1 copy

Note: The liquid reagent bottle contains slightly more reagent than indicated on the label. Please use pipette accurately measure and do proportional dilution.

Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator (CO2 incubator for cell culture is not recommended.)
3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)
4. Precision single (0.5-10μL, 5-50μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips(calibration is required before use.)
5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water

Sample Collection and Storage

1. Serum

Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20min at 1000xg and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

2. Plasma

EDTA-Na2/K2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future ′s assay. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. Tissue Sample

Generally tissue samples are required to be made into homogenization. Protocol is as below:

3.1. Place the target tissue on the ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Then weigh for usage.

3.2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS (e.g. 1mM PMSF).

3.3. Do further process using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

3.4. Homogenates are then centrifuged for 5 minutes at 5000×g. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future ′s assay.

3.5. Determine total protein concentration by BCA kit for further data analysis. Usually, total protein concentration for

Elisa assay should be within 1-3mg/ml. Some tissue samples such as liver, kidney, pancreas which containing a higher endogenous peroxidase concentration may react with TMB substrate causing false positivity. In that case, try to use 1% H2O2 for 15min inactivation and perform the assay again.

Notes: PBS buffer or the mild RIPA lysis can be used as lysates. While using RIPA lysis, make the PH=7.3. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT due to their severe inhibition for kits' working. We recommend using 50mM Tris+0.9%NaCL+0.1%SDS, PH7.3. You can prepare by yourself or contact us for purchasing.

4. Cell Culture Supernatant

Collect the supernatant: Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect clarified cell culture supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future 's assay.

Notes for Samples

Blood collection tubes should be disposable and non-endotoxin. Avoid to use hemolyzed and lipemia samples.

The best sample storage condition: less than 5 days at 2-8℃; within 6 months at -20℃; within 2 years at -80℃. Stored in liquid nitrogen for a longer storage. When melting frozen samples, rapid water bath at 15-25℃ can decrease the effect of ice crystal (0℃) on the sample. After melting, centrifuge to remove the precipitate, and then mix well.

The detection range of this kit is not equivalent to the concentration of analyze in the sample. For analyses with higher or lower concentration, please properly dilute or concentrate the sample.

4. Pretest is recommended for special samples without reference data to validate the validity.

Precautions for Kits

1. When using different Elisa kits, labeling is required to avoid mixed components and failed assay.

2. After opening the kit, please refer to the table of storage condition for coated plate and standards (Dampness may decrease the activity.).If any component is missing or damaged during the assay or storage, please contact us for ordering a new one to replace.(e.g. lyophilized standard)

3. Sterile and disposable tips are required during the assay. After use, the reagents bottle cap has to be tightened to avoid the microbial contamination and evaporation.

4. While manual washing, please keep tips or pipettors for adding wash buffer away from the well. Insufficient washing or contamination easily causes false positive and high background.

5. During the assay, prepare required reagents for next step in advance. After washing, add the reagent into the well in time to avoid dryness. Otherwise, dry plate will result in the failed assay.

6. Before confirmation, reagents from other batches or sources should not be used in this kit.

7. Don't reuse tips and tubes to avoid cross contamination.

8. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.

9. Please wear the lab coat, mask and gloves to protect yourself during the assay. Especially, for the detection of

blood or other body fluid samples, please follow regulations on safety protection of biological laboratory.

Recommended Sample Dilution Ratio

Please refer to shipped instructions or contact us for samples, dilution as well background info.

If other dilution ratio for your sample model is required, please refer to the universal dilution ratio below. (The ratio is suitable for single-well assay. For duplicate assay, please follow the calculation: volume of sample and diluent x number of duplicate well)

For 2 fold dilution (1/2): One step dilution. Add 60µL sample into 60µL sample diluent and mix gently.

For 5 fold dilution (1/5): One step dilution. Add 24µL sample into 96µL sample diluent and mix gently.

For 10 fold dilution (1/10): One step dilution. Add 12µL sample into 108µL sample diluent and mix gently.

For 20 fold dilution (1/20): One step dilution. Add 6µL sample into 114µL sample diluent and mix gently.

For 50 fold dilution (1/50): One step dilution. Add 3µL sample and 47µL normal saline (0.9% NaCl) into 100 µL sample diluent and mix gently.

For 100 fold dilution (1/100): One step dilution. Add 3µL sample and 177µL normal saline into 120µL sample diluent and mix gently.

For 1000 fold dilution (1/1000): Two step dilution. Create a 50-fold dilution first (normal saline is used throughout the dilution). Then, create a 20-fold dilution and mix gently.

For 10000 fold dilution (1/10000): Two step dilution. Create a 100-fold dilution first (normal saline is used throughout the dilution). Then, create the same dilution again and mix gently.

For 100000 fold dilution (1/100000): Three step dilution. Create a 50-fold dilution and 20-fold dilution respectively (normal saline is used in the first two steps.) Finally, create a 100-fold dilution and mix gently.

Notes: The volume in each dilution is not less than 3µL. Dilution factor should be within 100 fold. Mixing during dilution is required to avoid foaming.

Reagent Preparation and Storage

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25℃). For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

Assay Procedure Summary

Step1: Wash plate 2 times before adding Standard, Sample and Control (blank) wells!

Step2: Add 50ul Standard or Sample into each well. Immediately add 50ul Biotin-labeled Antibody into each well, gently tap the plate for 1min to ensure thorough mixing then static incubate for 45 minutes at 37°C.

Washing: Wash the plate three times and immerse for 1min each time.

Step 3: Add 100ul SABC working solution into each well, seal the plate and static incubate for 30 minutes at 37°C.

Washing: Wash the plate five times and immerse for 1min each time.

Step 4: Add 90ul TMB substrate solution, seal the plate and static incubate for 10-20 minutes at 37°C. (Accurate TMB visualization control is required.)

Step 5: Add 50ul stop solution. Read at 450nm immediately and calculate.

Detailed Assay Procedure

When diluting samples and reagents, they must be mixed completely. It's recommended to plot a standard curve for each test.

1. Set standard, pilot samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors. **Wash plate 2 times before adding standard, sample and control (blank) wells!**

2. Standards and samples loading: Aliquot 50ul of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube into each standard well. Also add 50ul sample dilution buffer into the control (blank) well. Then, add 50ul pilot samples into each sample well. Immediately add 50ul Biotin-labeled Antibody Working Solution into each well, gently tap the plate for 1min to ensure thorough mixing then static incubate for 45 minutes at 37°C. (Please keep tips or pipettors for adding Biotin-labeled Antibody away from the liquid level.)

3. Wash three times: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350ul wash buffer into each well and immerse for 1min. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step three times.

4. HRP-Streptavidin Conjugate (SABC): Add 100ul SABC working solution into each well. Seal the plate and static incubate for 30 minutes at 37°C. (Put the whole bottle of TMB into the 37°C incubator to equilibrate)

5. Wash five times: Remove the cover, and then wash the plate with wash buffer five times. Read washing method in step 3.

6. TMB Substrate: Add 90ul TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min. (Notes: Please do not use the reagent reservoirs used by HRP couplings. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells. Weaker or stronger color intensity is unacceptable.)

7. Stop: Keep the liquid in the well after staining. Add 50ul stop solution into each well. The color will turn yellow immediately. The order for adding stop solution and TMB substrate solution is the same.

8. OD Measurement: Read the O.D. absorbance at 450nm in a microplate reader immediately. (If your microplate reader has a choice of correction wavelength, set it to 570nm or 630nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. In this way, the OD value of non-chromogenic substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570nm or 630nm wavelength, the original OD450 value can be used.)

Calculation of Results

1. Calculate the mean OD450 value (using the original OD450 value or the corrected OD450 value) of the duplicate readings for each standard, control, and sample.
2. Create a four parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. Alternatively, you can use the curve fitting software offered by the microplate reader (e.g. Thermo SkanIt RE software, Curve Expert 1.3 or 1.4).
3. Calculate the sample concentration by substituting OD450 value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

Precision

Intra-assay Precision: samples with low, medium and high concentration are tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration are tested 20 times on three different plates.

Item	Intra-assay Precision	Intra-assay Precision	Intra-assay Precision	Inter-assay Precision	Inter-assay Precision	Inter-assay Precision
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	2.95	11.73	47.56	3.21	12.15	49.02
Standard deviation	0.14	0.55	2.72	0.16	0.55	2.90
CV(%)	4.9	4.71	5.71	4.96	4.51	5.91

Recovery

Add a certain amount of Pembrolizumab NAb into the sample. Calculate the recovery by comparing the measured value with the expected amount of Pembrolizumab NAb in the sample.

Matrix	Recovery Range (%)	Average (%)
Serum(n=10)	89-105	94
EDTA Plasma(n=10)	85-95	92
Heparin Plasma(n=10)	95-104	99

Linearity

Dilute the sample with a certain amount of Pembrolizumab NAb at 1:2, 1:4 and 1:8 to get the recovery range.

Matrix	1:2	1:4	1:8
Serum(n=10)	87-105%	89-101%	87-99%
EDTA Plasma(n=10)	89-104%	85-101%	87-93%
Heparin Plasma(n=10)	88-104%	89-99%	89-98%

Stability

Perform the stability test for the sealed kit at 37°C and 2-8°C and get relevant data.

Elisa kit(n=5)	37°C for 1 month	2-8°C for 6 months	2-8°C for 12 months
Average (%)	80	95-100	85-98

ELISA Troubleshooting

If the ELISA result is unsatisfied, please take a screenshot for the staining result and store the OD data. Keep used strips as well the rest reagents. Contact us to solve your problem with the kit's catalogue number and batch number. You can also refer to the following table to check the reason.

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect order for adding reagents	Confirm the required reagent added in each step. Also repeat the assay and verify.
Standard curve without signal	Use components from different kits	Use the component included in the same kit. Also repeat the assay and verify.
Standard curve without signal	Forget to add some reagents	Verify whether the required reagent is added.
Overflow OD	Use components from different kits, or prepare the working solution with higher concentration	Use the component included in the same kit. Also repeat the assay and verify.
Poor standard curve	Inappropriate curve fitting model	Try to plot the curve by different fitting models.
Samples without signal	The amount of pilot sample is lower than the detection range.	Decrease dilution ratio or concentrate the sample.
Samples without signal	The detection target is incompatible with the buffer.	Verify the compatibility of sample storage buffer with the pilot sample.
Samples without signal	Incorrect preparation of sample	Please refer to sample preparation guideline and regularly store.
Samples without signal	Longer storage of sample or freeze-thaw cycle	Aliquot and store samples according to the assay requirement.
High CV%	Precipitate is formed in the well during staining.	Increase the dilution ratio of the sample.
High CV%	Unclean plate	Don't touch the bottom of the plate during the assay.
High CV%	Foam is found in the well.	Avoid foaming during reading in a microplate reader.

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High CV%	Each well is washed unevenly.	Check whether the tube of the washer is smooth.
High CV%	Reagents are not completely mixed.	Mix all reagents completely.
High CV%	Inconsistent pipetting	Use calibrated pipette and correct pipetting method.
Standard curve with low signal	Standards are improperly reconstituted.	Before opening, shortly centrifuge the lyophilized standard tube till complete dissolution.
Standard curve with low signal	Standards have been degraded.	Follow suggested storage conditions for standards.
Standard curve with low signal	When pipetting, the required volume is incorrect or inaccurate.	Use calibrated pipette and correct pipetting method.
Standard curve with low signal	Expired kit	Don't use expired products.
Standard curve with low signal	Improper storage	Follow suggested storage conditions for all components.
Standard curve with low signal	The well is over dried.	The assay and sample loading process can't be terminated. Especially after washing the plate, add reagents immediately. Seal the plate during incubation.
Standard curve with low signal	Slow colorimetric reaction	Before use, equilibrate the whole bottle of TMB substrate for 30min at 37°C. Extend the incubation time.
Standard curve with low signal	The wavelength of the microplate reader is incorrect.	Check the wavelength and read the OD450 value again.
Standard curve with low signal	The well is washed excessively.	Follow suggested washing times in this manual.
High Background	Insufficient washing	Follow suggested washing times in this manual.
High Background	Wash buffer is contaminated.	Use the prepared wash buffer immediately. During manual washing, add wash buffer without touching the well.
High Background	Too many detection reagents or higher concentration.	Use calibrated pipette and correct pipetting method.
High Background	Reading of assay result is not in time.	Read the assay result immediately

		after adding the stop solution.
High Background	TMB substrate is incubated in strong light.	During colorimetry, incubate in the dark.

Declaration

Limited to current conditions and scientific techniques, all raw materials are not completely identified and analyzed. This product may have a technology-related quality risk.

During the Elisa kit development, some endogenous interferons(not all) in the biological sample have been removed or decreased.

The final assay result is related to the validity of reagents, experimental operation and environment. Our company is only responsible for this kit, excluding sample consumption during using this kit. Before use, please consider and prepare enough samples required by the assay.

To get a satisfied assay result, please use all reagents offered by this kit. Don't use any product from other vendors. Strictly follow instructions of this manual.

During assay procedure, incorrect reagents preparation and parameter setting of the microplate reader may result in the abnormal result. Before assay, please read this manual carefully and adjust instruments.

Even if the assay is performed by the same person, results in two independent assays may be different. Thus, each step in the assay should be controlled to ensure the reproducibility.

Before delivery, this kit is subject to the strict QC. Influenced by transportation conditions and experimental devices, the assay result got by the customer may be different from original data. Inter-assay CV between different batches may be caused by reasons before.

This kit is not compared to similar kits from other vendors or methods for testing the same detection target. Thus, assay results may be inconsistent.

This kit allows for research use only. For IVD or other purposes, our company is not responsible for relevant consequences and doesn't bear any legal liability.