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(For scientific research use only, not for clinical diagnosis!)

Human programmed death molecule-1 antibody (PD-1 Ab) quantitative detection kit (ELISA) instruction manual Specifications: 48T/96T

Please read the instructions carefully before use. If you have any questions, please contact us through the following methods: Official hotline: 400-999-8863 Technical phone: 18358180525 Email: Upingbio.163.com Company website: www.upingbio.com For the specific shelf life, please see the outer packaging label of the kit. Please use the kit within the shelf life.

When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.

Kit performance Physical properties: Each liquid component is clear and transparent, with no precipitation or floc. Microplate aluminum foil bags should be vacuum packed without damage or leakage.

Standard curve linearity: Calibrator dose-response curve correlation coefficient r value, greater than or equal to 0.9900.

Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.

Sensitivity: The lowest detectable dose is less than 0.1 ng/mL.

Recovery rate: The recovery rate is between 85%-115%.

Sensitivity: This kit recognizes natural and recombinant human programmed death molecule-1 antibodies (PD-1 Ab) and has no crossover with structural analogs.

Stability: Stored at 2℃-8℃, validity period is 6 months.

Detection range: 0.25 ng/mL – 8 ng/mL.

Purpose: Used to detect the concentration of human programmed death molecule-1 antibody (PD-1 Ab) in serum, plasma, cell culture supernatant and other samples.

Experimental principle This kit uses an indirect two-step enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with human programmed death molecule-1 protein (solid-phase antigen), add different concentrations of calibrators and samples to be tested, and after incubation and sufficient washing, unbound components are removed, and then Add another strain of HRP-labeled anti-human Ig-HRP (enzyme-labeled antibody), and after incubation and sufficient washing, unbound components are removed to form a solid-phase antigen-antibody-enzyme-labeled antibody structure on the solid surface of the microplate.

Sandwich compound. Add substrates A and B. Under the catalysis of HRP, the substrate produces a blue product. Under the action of the stop solution (2M sulfuric acid), it is finally converted into yellow. The absorbance (OD value) is measured on a microplate reader. The absorbance (OD value)) is positively correlated with the concentration of the antibody to be tested in the sample to be tested. Ready for school

The standard curve can calculate the concentration of the antibody to be tested in the sample.

Kit components and storage: Store unopened kits at 2-8

degrees Celsius. Do not use expired kits.

Components	quantity	Main ingredients
Calibrator	0.3ml/tube*6 tubes	Antibody preparations in 6 concentration standards
coated microplate	96T	Pre-coated solid phase antigen
HRP labeled antibodies	10mL	HRP-labeled detection antibodies
Substrate solution A	6mL	carbamide peroxide working solution
Substrate solution B	6mL	TMB working fluid
sample diluent	60mL	PBS
stop solution	6mL	2mol/L dilute sulfuric acid
20×concentrated washing liquid	30mL	PBS with 0.15% Tween20
manual	1 serving	--
ziplock bag	1	--
Self-adhesive	2 tablets	--

The concentrations of calibrators are: 8, 4, 2, 1, 0.5, 0 ng/mL.

Note: 1: Before use, please check whether the label and quantity of

the reagents in the kit are consistent with the table.

2: If the components of the kit need to be used again, please ensure that they have

not been contaminated since the last use. 3: If the enzyme plate is not used up in a

single time, remember to seal it and store it at 2-8°C.

Self-prepared test equipment required for the test

(not provided, but can be purchased with

assistance) 1. Standard specification microplate

reader.

2. Automatic plate washing machine.

3. Oscillator.

4. A series of adjustable pipettes and tips. When testing a large number of samples at one time, it is best to use a multi-channel pipette. 5. Dilute 25×TBS-T wash buffer 25 times with deionized water to 1×TBS-T wash buffer.

Restrictions on the kit: 1. For scientific

research use only and not for clinical

diagnosis.

2. Use within the validity period marked on the kit. Expired products must not be used.

3. Do not mix with kits or components from other manufacturers.

4. Use the sample diluent provided with the kit.

5. If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then re-measure. 6. Human anti-mouse and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

7. The test results obtained by other methods are not directly comparable to the test results of this kit.

Notes: 1) This kit is for in vitro research only and

not for clinical diagnosis.

2) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

3) Carry out incubation strictly according to the specified time and temperature to ensure accurate results. All reagents must come to room temperature before use

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20-25℃. Store reagents refrigerated immediately after use.

4) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.

5) Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.

6) The substrate chromogenic solution should be colorless or very light in color.

7) Avoid cross-contamination of reagents and specimens to avoid erroneous results.

8) Avoid direct exposure to strong light during storage and incubation.

9) Equilibrate to room temperature before opening the sealed bag to prevent water droplets from condensing on the cold slats.

10) Any reaction reagents must not come into contact with bleaching solvents or strong gases emitted by bleaching solvents. Any bleaching ingredients will destroy the biological activity of the reagents in the kit.

11) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of 450±10nm, and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.

12) Do not mix or replace the reagents in this kit with reagents from other batch numbers or other sources.

13) The EP tubes and tips used in the test are single-use and are strictly prohibited from mixing.

14) Do not use expired reagents.

Sample preparation and storage

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide must not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

Cell culture supernatant - centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.

Serum - Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.

Plasma—anticoagulate with heparin, citrate, or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood draw. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

Cell lysis buffer - For adherent cells, remove the culture medium and wash with PBS, normal saline or serum-free culture medium. Add an appropriate amount of lysis solution and pipet several times with a gun to fully contact the lysate and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, blow the cells with a gun, and flick them with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

Urine - Collect in sterile tubes and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.

Reagent preparation 1. Before use, all components must be rewarmed for at least 120 minutes to ensure sufficient rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Dilute concentrated washing liquid and distilled water at 1:20, that is, add 1 part of concentrated washing liquid to 19 parts of distilled water.

3. Substrate: Substrate solutions A and B, mix thoroughly at a volume of 1:1 before use, and use within 15 minutes after mixing.

Operating procedures

Recommended sample dilution scheme: It is recommended that teachers conduct preliminary experiments to explore the optimal dilution ratio of samples before conducting formal experiments.

1. Return all reagents and components to room temperature first. It is recommended to perform duplicate holes for standards, quality control materials and samples.

2. Prepare working solutions for various components of the kit according to the method described in the previous reagent preparation.

3. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.

4. Set up standard wells, sample wells and blank wells. Add 50 μL of standards of different concentrations to each of the standard wells. Add 50 μL of the sample to be tested to the sample wells. Do not add any to the blank wells. Cover the reaction plate with a sealing film and place in a 37°C water bath. Incubate in a pot or incubator for 30 minutes.

5. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing solution, let stand for 20 seconds, shake off the washing solution, and pat dry on absorbent paper. Repeat this 4 times (wash the plate 5 times in total). If you use an automatic plate washer, please wash the plate according to the operating procedures of the plate washer. Adding a soaking program for 20 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper.

6. In addition to the blank wells, add 100 μL of horseradish peroxidase-labeled detection antibody to the standard wells and sample wells.

7. Cover the reaction plate with sealing film and incubate it in a 37°C water bath or incubator for 30 minutes.

8. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing solution, let stand for 20 seconds, shake off the washing solution, and pat dry on absorbent paper. Repeat this 4 times (wash the plate 5 times in total). If you use an automatic plate washer, please wash

the plate according to the operating procedures of the plate washer. Adding a soaking program for 20 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper.

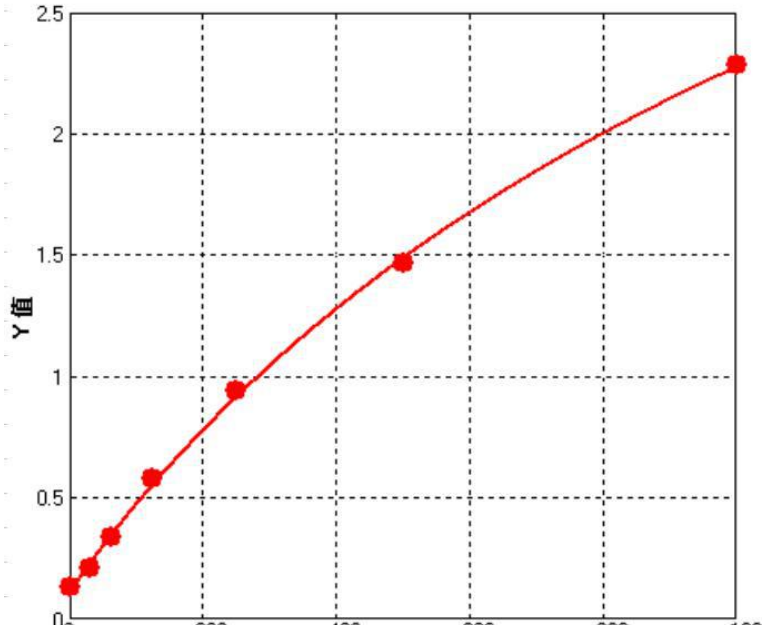
9. Mix substrates A and B thoroughly at a volume of 1:1, and add 100 μL of substrate mixture to all wells. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator for 15 minutes.

10. Add 50 μL of stop solution to all wells, and read the absorbance (OD value) of each well at a wavelength of 450 nm on a microplate reader.

Result calculation

11. Use the concentration of the standard substance as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculation using ELISA Calc software]

12. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample.



(Schematic diagram, for reference only)

[Problem Analysis] If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the used strips and unused reagents, and then contact our company's technical support to solve the problem for you. At the same time, you can also refer to the following information: [Questions and Answers]

Problem Description	Possible Causes	Corresponding countermeasures Corresponding countermeasures
standard curve gradient difference	Incorrect liquid aspiration or	Check pipettes and tips
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washings and the amount of liquid
Very weak or colorless	Incubation time too short	Ensure adequate incubation time
	The experimental temperature is incorrect	Use recommended experimental temperatures
	Insufficient reagent volume or missing addition	Check the liquid aspirating and adding process to ensure that all reagents are added in order and in
	Incorrect dilution	
	Enzyme label inactivation or substrate failure	Mix enzyme conjugate and substrate and check by rapid color development
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter
		Turn on the microplate reader and preheat it in advance
Large coefficient of variation	Adding fluid incorrectly	Check the filling situation
High background value	The working concentration of the	Use the recommended dilution
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked;
	The lotion is contaminated	Prepare fresh lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to

[statement]

1. Limited by the existing conditions and scientific and technological level, it is not possible to conduct comprehensive identification and analysis of all raw materials. This product may have certain quality and technical risks.

2. The final experimental results are closely related to factors such as the effectiveness of the reagents, the relevant operations of the experimenter, and the experimental environment at the time. Our company is only responsible for the kit itself and is not responsible for the sample consumption caused by the use of the kit. Please use The user should fully consider the possible usage of the sample and reserve sufficient samples before use.

3. In order to achieve good experimental results, please only use the reagents provided in our company's kits, do not mix products from other manufacturers, and operate in strict accordance with the instructions.

4. Due to incorrect reagent preparation and microplate reader parameter settings during the operation, abnormal results may result. Please read the instructions carefully and adjust the instrument before the experiment.

5. Even if operated by the same personnel, different results may be obtained in two independent experiments. In order to ensure the reproducibility of the results, it is necessary to control every step of the experimental process.

6. The kits will undergo strict quality inspection before shipment. However, due to factors such as transportation conditions, differences in experimental equipment, etc., user test results may be inconsistent with factory data. Differences between kits from different batches may also result from the above reasons.

7. This kit has not been compared with similar kits from other manufacturers or products that detect the same target substance using different methods, so inconsistent test results cannot be ruled out.

8. The kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company will not be responsible for any problems arising therefrom, nor will we assume any legal liability.

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